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THE UNIVERSITY OF ALBERTA

CHARACTERISTICS OF RECOMBINATION AT THE *SUP6* LOCUS IN *SACCHAROMYCES CEREVISIAE*

by

LAURA L. DiCAPRIO ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA

FALL, 1976

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled, "Characteristics of recombination at the *SUP6* locus in *Saccharomyces cerevisiae*," submitted by Laura L. DiCaprio in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Spontaneous secondary mutations of *SUP6*, a tyrosine-inserting ochre suppressor, were selected in a haploid strain of *Saccharomyces cerevisiae*. Allelic recombination within the locus was studied by meiotic analysis of random spores as well as by gamma ray induction of mitotic recombination in all possible crosses of ten strains with mutant suppressors and a wild-type $sup6^+$ strain. Recombinants were selected by selecting for restoration of the suppressor phenotype in strains with suppressible auxotrophic requirements. Meiotic analysis revealed the following characteristics of recombination at this locus.

1. The recombination frequencies in two-point crosses were unsatisfactory as a criterion for mapping alleles within *SUP6*. However, a mathematical technique was devised for using meiotic recombination frequencies to obtain a map. The rates of gamma ray-induced mitotic recombination also proved to be unsatisfactory for mapping.
2. One of the two recombinant outside marker combinations was recovered more often than the other among selected intragenic recombinants in two-point crosses. Both classical crossover theory and current models of recombination predict that the allele order is that which makes the more common class the single exchange class. This criterion was used to determine an order for *SUP6* alleles which was internally consistent.
3. The selected intragenic recombinants with outside markers in the parental configuration showed no evidence for a polarity of conversion in this locus, that is, no gradient of conversion from one end of the locus to the other.

4. There existed allele-specific and heteroallelic-combination-specific effects on intragenic recombination which could not be accounted for solely by the correction properties of the alleles.
5. Among selected prototrophs, there were more products with the parental configuration of outside markers than the recombinant configuration. This was true for all allele combinations and the difference was significant for most crosses.

Tetrad analysis of one-point crosses of *SUP6* (either *SUP6-1* x *sup6*$^+$ or *SUP6-1* x *sup6-1-x*) showed that for two of three *SUP6* alleles tested, conversion was accompanied by outside marker recombination less than 50% of the time. This excess of parental over recombinant products confirmed the observation with selected recombinants in two-point crosses. A high frequency of conversion and very long conversion lengths, one-third of which extended into an adjacent marked locus, were also revealed by tetrad dissection. The conversion events predominantly involved one chromatid only, although there were probably some two-chromatid events, and some apparent four-chromatid events. Conversion on two pairs of chromatids occurred more frequently than predicted from the frequency of conversion events involving a single pair of non-sister chromatids. Independent conversion of linked loci showed no positive or negative interference and there was some indication of a lack of interference between crossovers in the region. The high conversion frequency accompanied by a low recombination frequency between *SUP6* alleles indicated that most conversion events covered the entire locus and did not stop between the alleles.

The presence of dimer excision-repair defects in strains carrying *rad1*, *rad2*, *rad3*, or *rad4* mutations or the presence of other repair defects caused by *rad16* and *rad18* mutations did not drastically alter the pattern of conversion from that in wild-type strains. In particular, no changes in the frequency of correction of mismatched bases in meiotic recombination (seen as postmeiotic segregation) was noted.

ACKNOWLEDGEMENTS

These studies were completed with the assistance of several people. I would like to acknowledge the help I received from Philip Hastings, particularly in the analysis and interpretation of results. Also, I thank Kenneth Morgan who took an interest in the mapping problem and spent a considerable amount of time on the quantitative analysis of the data. Mickey Tan who drafted the diagrams and graphs and Ralph DiCaprio who helped with the computer analysis are also thanked for their contributions. And I owe a great deal to the help I received from the other students in the laboratory, particularly from Donald Morrison who always took an interest in the progress of my laboratory work.

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$\frac{\textit{his2} \quad \textit{cdc14} \quad + \quad +}{+ \quad + \quad \textit{SUP6-1} \quad \textit{met10}}$ or $\frac{\textit{his2} \quad \textit{cdc14} \quad \textit{sup6-1-x} \quad +}{+ \quad + \quad \textit{SUP6-1} \quad \textit{met10}}$ 131

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INTRODUCTION

Early descriptive work on the genetic consequences of recombination within a locus revealed certain patterns which provided the framework for general model building. The picture of eukaryote recombination which emerged involved the formation of hybrid DNA as a result of base pairing between complementary nucleotide chains from DNA molecules of different parental origin. The exchange was thought to be initiated at a fixed point at the end of a locus. Inclusion of a mutation in this hybrid region would result in the formation of a mismatched base pair, and it was postulated that an enzymatic repair system could recognize the mismatch and correct it by excising and replacing the mismatched base on one strand. Reciprocal recombination of outside markers could follow from resolution of such a half-chromatid exchange.

The diagrams in Figure 1A of the Holliday (1964) model of recombination illustrate one way in which this sequence of events could take place. Markers are included so the genetic consequences of such events may be seen. The interpretation of the results presented in this study is based on a general model of recombination through hybrid DNA formation and mismatch correction, and the differences between models of this kind are generally not relevant. However, Figure 1B (adapted from Meselson and Radding (1975)) shows a configuration of a recombinant molecule with hybrid DNA of unequal length on the two chromatids as well as the capacity for migration of the crossconnection in either direction. This illustrates the large potential for variation in the characteristics of an event as different parameters are allowed to vary independently.

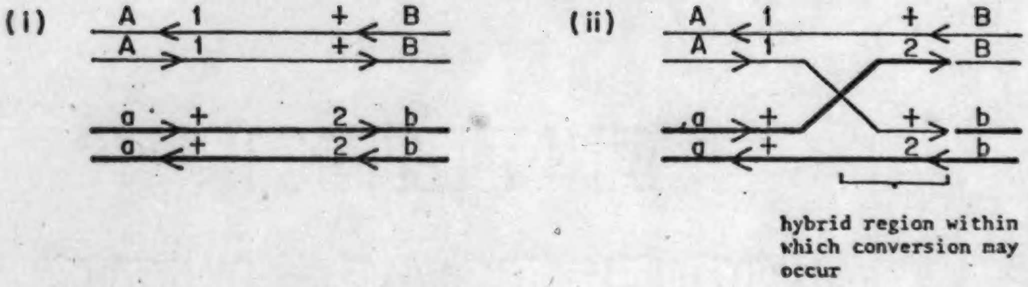
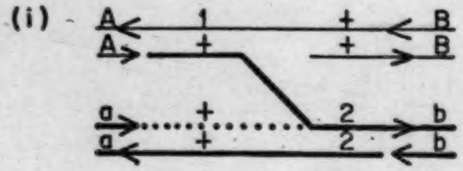
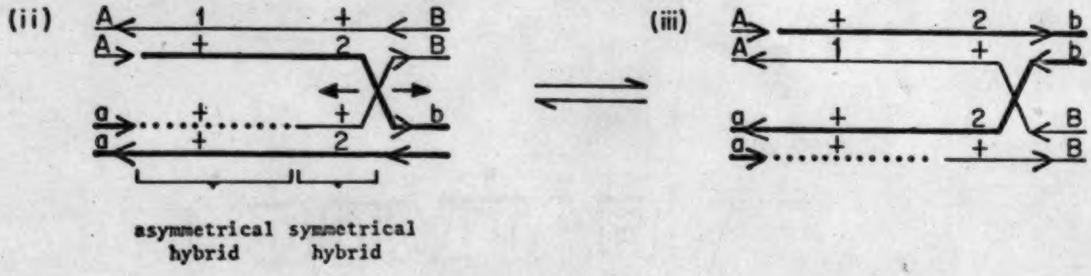

(i)
A 1 + B
A 1 + B
a + 2 b
a + 2 b
(ii)
A 1 + B
A 1 2 B
a + + b
a + 2 b
hybrid region within which conversion may occur

Figure 1. Diagrams of recombination mechanisms.

A. (i) Before recombination. Each chromatid involved in the recombination event is represented by two lines of opposite polarity, believed to be the nucleotide chains of a DNA molecule.

(ii) Breaking and rejoining of the two crossed nucleotide chains will give a product with the outside markers in the parental configuration. Scission of the outside strands will yield a product recombinant for markers A and B. The mismatch created by hybrid DNA covering the site of allele 2 may be excised and complementary bases inserted on one strand for one or both pairs of hybrid DNA molecules.





B. The configuration before recombination is the same as in A.

(i) The recombination event is initiated asymmetrically. A single-strand break in one DNA molecule results in that strand pairing with the complementary base sequence in another DNA molecule while DNA polymerase activity displaces the strand which initiated the event. The dotted line represents a region of new DNA synthesis. Hybrid DNA is asymmetrical, that is, restricted to one chromatid.

(ii) and (iii) Rearrangement of configuration (i) may result in the formation of symmetrical hybrid (reciprocal formation of hybrid on the DNA molecules of both chromatids). Since the position of the outside and the bridging strands can be interchanged in the model by rotation without bond breakage, configurations (ii) and (iii) are believed to be in rapid equilibrium. One will result in products with the outside markers in the parental configuration, the other will give recombination of outside markers.

Evidence that intragenic recombination is not predominantly a consequence of crossing-over between alleles came from studies in Ascomycetes, in which the products of meiosis are held together in the ascus. The isolation of individual asci from mutant x wild-type crosses occasionally gave 3 wild-type and 1 mutant spore or 3 mutant and 1 wild-type, rather than the expected 2:2 segregation (Zickler 1934; Lindegren 1953; Michell 1955). In studies of conversion which included markers on either side of the locus being analysed, it was shown that allelic recombination resulting from conversion of one allele in a two-point cross was highly correlated with recombination of outside marker genes (Stadler and Towe 1963). This was true of conversion in one-point crosses as well (Kitani et al. 1962). Also, a large number of studies of random meiotic products in which intragenic recombinants were selected showed a high association of intragenic recombination with recombination of the outside markers. Small samples of tetrads with recombinant products from these same systems showed conversion to be responsible for much or all of the allelic recombination (for example, Case and Giles 1958b). The high association between conversion and crossing-over was an important consideration in model building.

Another body of data which had to be accounted for was the property that a set of alleles could often be mapped using the classical criterion of the frequency of recombination between them. Crosses were performed between strains each carrying a different auxotrophic mutation in the same locus and prototrophs were selected among the meiotic products. The distances, measured in terms of recombination frequencies, were often reasonably additive, and thus it appeared as though allelic recombination involved some distance-dependent parameters.

On the basis of hybrid DNA models, the production of an allelic recombinant requires that the segment of the chromatid in which conversion occurs cover one site and not the other. Distance-dependency indicated that the probability of a conversion length ending between two sites increases with greater separation of the sites, since conversions covering both sites will not result in the formation of a recombinant. The models provide for two distance-dependent components of recombination, hybrid DNA endings and excision-repair endings. If hybrid DNA is initiated at a fixed point and is variable in length, then the probability that one site will be covered by hybrid but not the other will depend on the distance between the sites.

In addition, the phenomenon of co-conversion, that is, conversion of more than one allele in a gene in the same direction on the same strand, indicated that correction takes place over a length rather than at a point (Case and Giles 1958a, 1964; Rizet and Rossignol 1963; Fo[illegible] and Mortimer 1969). Since the ending of a segment of excision and resynthesis between two alleles may also generate a recombinant even if both alleles are included in the same hybrid segment, the excision endings are a second distance-dependent parameter contributing to recombination.

Fincham and Holliday (1970) showed how the properties of these two components of recombination could explain a certain characteristic of fine-structure mapping, the phenomenon of map expansion (Holliday 1964). This is a term given to the common observation in intragenic mapping that the recombination frequency between two widely-spaced mutants exceeds the frequency obtained by adding the recombination frequencies of more closely spaced mutants in the same interval. For the map order a-b-c, a-c > a-b + b-c. Mutants closer than the average excision length, if included in a single hybrid region, would generally be co-converted and thus generate no recombinants. In such a situation, recombinants could arise only from hybrid DNA ending between the alleles. At greater distances, correction could include one allele but not the other, and thus both hybrid and excision endings would contribute significantly to the production of recombinants, resulting in expansion of longer segments.

It must be noted that not all loci studied have been found to be mappable by recombination frequency in two-point crosses. Kruszewska and Gajewski (1967) and Paszewski et al. (1971), working with *Ascobolus immersus*, showed that there were factors other than the distance between two alleles which determined the frequency with which one site was converted but not the other. It was seen that the presence of a second allele in two-point crosses affected the occurrence or the properties of the recombination events. Stadler and Kariya (1969), in a study of the *mtr* locus of *Neurospora crassa*, found that inclusion of an additional site of heterozygosity increased conversion and decreased the recombination frequency between two alleles spanning this site. They explained this as the result of a nearby mismatch extending the correction length. Hastings (1975) interpreted this and several other marker effects as a change in the distribution of hybrid DNA. Any of these interpretations provides for distance-independent factors which contribute to recombination frequency, and the extent of their contribution will determine the mappability of a locus.

Another mapping criterion depends on the segregation of outside markers among selected intragenic recombinants. In a cross of the form A1+B x a+2b, a++B is expected to be the more common class of prototroph with outside markers recombined. Classically, this genotype results from a single crossover, and in terms of recombination as a process of conversion and reciprocal exchange, it is the product expected from conversion of either allele accompanied by an adjacent crossover. The reciprocal class A++b requires hybrid DNA to have covered both sites with independent correction of the two alleles. Since this type of event will generate both classes of recombinants and hybrid covering one allele can generate only the single exchange type, the single exchange should be the more common product. In a tetrad it would be seen as a crossover separated from the site of conversion by a 2:2 segregation of the other allele to give the tetrad : A1+b/A++b/a+2B/a+2b. There are examples in *Neurospora*, however, in which the near-equality of the two recombinant classes makes mapping by this criterion impossible (Fincham 1967; Stadler and Kariya 1969).

The concept of a fixed breakage point at which the formation of hybrid DNA is initiated arose from studies of allelic recombination between white-spored mutants of *Ascobolus immersus*. The work reported by Lissouba et al. (1962) for two-point crosses of mutants in series *46* showed that all intragenic recombinants in this locus arose from conversion of the site to the right. The preferential conversion of the more distal or the more proximal allele in two-point crosses sometimes seen in tetrads is explained by a gradient of conversion from one end of the locus to the other, and is referred to as polarity.

A conversion gradient also explains the frequently observed inequality of the two parental classes of outside markers among selected random meiotic products (for example: Siddiqui 1962; Murray 1963). The parental arrangement of outside markers which entered the cross with the distal allele is consistently the more common among selected intragenic recombinants in some loci and the parent which entered with the proximal allele is the more frequently recovered in other systems. This suggests that when one allele is converted it is always the one to the left (or right).

Further confirmation of a fixed point of initiation comes from the work of Angel, Austin and Catcheside (1970) on the control of recombination in *Neurospora*. This work involves the discovery and description of the *cog* locus which appears to be a region in which recombination in the closely-linked *his-3* locus is initiated.

There are other conversion phenomena which could only have been revealed by tetrad analysis, for example, postmeiotic segregation. Postmeiotic segregation was first observed as the segregation of spore-color markers in the first mitotic division after meiosis in *Sordaria fimicola* (Olive 1959). This phenomenon was later seen in *Neurospora crassa* (Stadler and Towe 1963; Case and Giles 1964) and in *Ascobolus immersus* (Lissouba et al. 1962). The observation of 3:5 and 5:3 segregations in 8 spored asci means that there were differences in the two sister spores which came from the first mitotic division of a meiotic product. Conversion appears to have affected only half a meiotic product in those cases in which postmeiotic segregation has occurred. This suggests that chromatids have a two-stranded nature and that the two strands may differ at the end of Meiosis II. In the general recombination model presented, these strands are the nucleotide strands of a hybrid DNA molecule.

The work of Leblon and Rossignol (1973) supports the idea that postmeiotic segregation results from an uncorrected heteroduplex intermediate in recombination and that conversion is a process of correction of heterozygosity. They observed that an allele with a high frequency of postmeiotic segregation in one-point crosses showed decreased postmeiotic segregation when that mutation was coupled with one which had a characteristically low postmeiotic segregation frequency. These observations, interpreted in terms of mismatch repair, suggest that correction of the first mismatch took place under the influence of the second. By this interpretation, it appears that correction is induced by a heterozygous site in a hybrid DNA molecule and that correction may start at one site and extend to another.

As was mentioned earlier, recombination models as they are now presented allow for a great deal of flexibility and variation (Sobell 1972; Meselson and Radding 1975; Wagner and Radman 1975). Migration of the hybrid region and sections of symmetrical hybrid (hybrid DNA on both chromatids) and asymmetrical hybrid (hybrid DNA on one chromatid only) make it possible to accommodate the large amount of variation in patterns of recombination in different organisms and in different loci in the same organism. For example, the observation that the proportion of events which involve one chromatid only rather than symmetrical two-chromatid events varies from one system to another (Stadler and Towe 1971; Leblon and Rossignol 1973; Kitani and Whitehouse 1974a) is compatible with any one of these models.

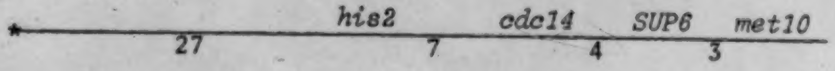
Since flexibility in models of recombination is necessary to accommodate the extensive variation in the characteristics of recombination, fine structure mapping does not have as its objective the restriction of different parameters, for example, the amount of hybrid migration, the probability and length of excision, or the amount of asymmetrical and symmetrical hybrid, in order to define the universal recombination event more exactly. What may be sorted out by comparative studies are the properties characteristic of an organism's enzyme system, those affected by the specific base sequence or the amount of heterozygosity in the region being studied, and those dependent on controlling elements of a gene. Some parameters may be free to vary independently and others will be dependent on each other or under common control. When the characteristics of recombination in an organism are familiar, the absence of an expected pattern may lead to the separation of other factors which have modified its expression. For example, in *Ascobolus immersus*, the absence of polarity in gene *75* (Rossignol 1969) revealed allele-specific effects on conversion frequencies which masked polarity.

In addition, although the quantitative properties of different parameters of recombination are expected to differ in different systems, the descriptive genetic analysis of recombination does offer some information pertinent to the choice of one recombination model over another. For example, in both the Sobell (1972) and Wagner and Radman (1975) models, the initiation of recombination is a symmetrical event involving two chromatids equally. The results of Angel, Austin and Catcheside (1970) and Catcheside and Angel (1974) indicate very strongly that at least in *Neurospora*, initiation is not necessarily symmetrical.

cog, the region which appears responsible for the initiation of recombination in the closely-linked *his-3* locus may exist in an active or inactive form. Recombination in a heterozygous *cog*/*cog*$^+$ strain is initiated on the chromatid carrying the active *cog*$^+$ allele and, furthermore, conversion of point mutations in *his-3* appears to be preferentially on the strand containing the active initiation region.

There are a limited number of studies of meiotic intragenic recombination in *Saccharomyces cerevisiae*. Because of the amount of variation possible in different loci within an organism, one must be cautious about generalizing about mechanisms of recombination even in the one species. This study of the *SUP6* locus was undertaken with the knowledge of its high frequency of conversion (11% in the study of Hurst, Fogel and Mortimer (1972)). *SUP6-1* is a super-suppressor mutation which causes the insertion of tyrosine at the position specified by the ochre nonsense codon UAA. (Gilmore, Stewart and Sherman 1971). Because of the properties of this type of nonsense suppressor mutation in yeast, it was expected that these loci specified the production of tRNA molecules. However, only recently has it been shown that yeast suppressors of UAA (ochre) and UAG (amber) nonsense codons can suppress termination in an *in vitro* translation system and that this suppression is mediated by purified tRNA from suppressing yeast strains (Capecchi, Hughes and Wahl 1975; Gesteland et al. 1976). The original *SUP6* mutation to an ochre suppressor is thought to be in the anticodon of the tRNA, and therefore the wild-type allele at this position was designated as AC in this study. However, there is no direct evidence for this assumption. A discussion of the indirect evidence is found in a review article by Hawthorne and Leupold

(1974). *SUP6* is located on linkage group VI and has the following linkage relationship to the other markers used in this study (Mortimer and Hawthorne 1973). Distances are given in percent recombination.



The questions asked using this system were general ones, whether there were characteristics peculiar to a region of high conversion, what properties this locus had in common with others in this organism, how much heterozygosity and base sequence contributed to the high conversion properties of this region, and whether this could be developed as a system suitable for the study of allele-specific effects on different parameters of recombination. Secondary mutations of *SUP6-1* which had lost suppressor activity were easily selected. With these one could test whether the high conversion frequency and other characteristics of the locus were allele-specific effects of the original *SUP6-1* mutation or properties of the locus. This was done by tetrad dissection of crosses of *SUP6-1* by *sup6-1-x* (a mutation of the suppressor). In addition, crosses homozygous for *SUP6-1* or for $sup6^{+}$ (the wild-type nonsuppressing allele) were performed in order to test whether heterozygosity at the suppressor locus was having an effect on conversion at nearby loci. Pairwise crosses of secondary mutations of the suppressor were also made in the hopes of studying allelic recombination by tetrad dissection. However, the low frequency of recombination made it necessary to study allelic recombination by selection of recombinant random meiotic products. The information from these studies made it possible to compare the properties of *SUP6* with those of other loci, properties often used in model building and selection.

There is another approach to the study of recombination which has been discussed by Radding (1973) and Clark (1974). This involves treating recombination as a metabolic process which may follow several different pathways that overlap extensively with replication, repair and transcription. The amount of overlap is not a constant, but varies from organism to organism. One method of studying this overlap is to look for pleiotropic effects of recombination- and repair-defective mutations.

Several mutations in the dimer excision-repair pathway of *Saccharomyces cerevisiae* were analysed in *SUP6*-carrying strains by tetrad dissection. Postmeiotic segregation, thought to result from uncorrected mismatches, is easily recognized in this system because of the red pigment produced by adenine-requiring cells. The excision of pyrimidine dimers from UV irradiated DNA is often used as an analogy for conversion as a process of mismatch repair. Although there is some evidence in prokaryotes for an interaction between dimer removal and presumed mismatch excision in transformation and phage recombination systems (Doerfler and Hogness 1968; Bresler et al. 1971), the two processes are probably only indirectly related, and thus not mediated by the same set of enzymes (Lacks 1970; Spatz and Trautner 1970; Guild and Shoemaker 1974). By checking whether conversion events were lost to postmeiotic segregations in these repair-defective strains, it would be seen whether the two kinds of excision share common steps in *Saccharomyces cerevisiae*.

Two additional radiation-sensitive mutations which are thought to block first steps in other repair pathways were tested. The loss of one step in the process of recombination if other alternatives are available may only change the proportions or characteristics of the events without blocking them entirely. Even in instances of shared steps then, repair defects might have only subtle effects on recombination. However, these should be detectable in a tetrad dissection system capable of revealing the length, frequency and distribution of conversions and crossovers.

MATERIALS AND METHODS

Media

YEPD: 1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose (2% Bacto-agar).

.1 YEPD: Yeast extract reduced from 1% to .1%. All other ingredients as in YEPD.

YEPG: 1% Bacto-yeast extract, 2% Bacto-peptone, 3% glycerol, 2% Bacto-agar. Used to test for respiratory sufficiency.

Defined medium: .67% Bacto-yeast nitrogen base without amino acids, 2% dextrose (2% Bacto-agar). Adenine, arginine, lysine, histidine, methionine, tryptophan and uracil 20 mg; leucine 30 mg; threonine 350 mg in a total of 100 ml stock solution added to a liter of medium.

Omission media: Defined medium lacking one or more of the above supplements.

-MTh: double omission medium lacking methionine and threonine, used to score *met10-4*.

-ALT: triple omission medium lacking adenine, lysine and tryptophan, used to select for functional suppressors in haploid strains.

-LT: double omission medium lacking lysine and tryptophan, used to select for functional suppressors in diploid strains.

Canavanine medium: Arginineless omission medium plus 60 or 200 mg filter sterilized canavanine sulfate.

Sporulation media:

Liquid: 1% potassium acetate.

Solid: 1% potassium acetate, .25% Bacto-yeast extract, 2% Bacto-agar. Supplemented as for defined medium.

Strains

The origin and genotypes of the stocks used to construct all strains used in this study are listed in Table 1. The array of crosses performed is shown in Figure 1, and Table 2 lists the genotypes of the haploids given in this figure and referred to in the text.

Selection of Secondary Mutations of *SUP6-1*

The haploid strain A14-2B (a, *ade2-1*, *lys1-1*, *trp5-48*, *can1-100*, *SUP6-1*, *met10-4*, *ura4-11*) was grown in liquid defined medium lacking adenine, lysine and tryptophan to maintain selection for the suppressor. Then samples were put into tubes of liquid YEPD, allowed to reach stationary phase, and plated on canavanine medium (200 mg/l). Canavanine resistant colonies were picked, one per tube, and those showing a strict requirement for adenine, lysine and tryptophan were used for further study.

Sporulation and Dissection

When meiotic products were to be dissected, the haploid parents were mixed on solid YEPD and, wherever possible, replica plated to a multiple omission medium selecting for diploids. These were transferred to solid sporulation medium and left at 25° for four days or longer before dissection. When selection of diploids was not possible, single zygotes were usually picked by micromanipulation from the mating mixture, allowed to grow, then sporulated as above. Asci were digested with a one in twenty dilution of glusulase (Endo) at 37° for 15—30 minutes, then dissected with a De Fonbrune micromanipulator directly onto YEPD plates. In later experiments, .1 YEPD was used to facilitate development

TABLE 1

Genotypes and origin of stocks used to construct strains for this study.

X 3417-32C:	a; *asp5-1, trp5-48, arg4-0, lys1-1, his2, cdc14, SUP6-1, met10-4, trp1, ade1* (Mortimer)
1403:	α; *ura4-11* (Magni)
XV 162-2C:	a; *trp5-48, arg4-17, lys1-1, ade2-1, hom3-10* (von Borstel)
X 2280-4A:	α; *trp5-48, his5-2, lys1-1, ade2-1, ura3-1, met, can1-100, SUP6-1* (Mortimer)
X 932-4A:	a; *ser*$^-$ (Mortimer)
197/2D:	α; *rad*$^-$, *ade2-1* (Cox)
133/3A:	α; *rad18-1* (Game)
uvs 1-2:	α; *rad1-18* (Resnick)

TABLE 2

. Genotypes of strains shown in Appendix I.

A 1-1:	α; *ade2-1, lys1-1, trp5-48*
A 2-1:	*ade2-1, trp5-48, can1-100*
A 3-1:	*ade2-1, lys1-1, trp5-48, can1-100, leu2*[1]
A 3-2:	α; *ade2-1, lys1-1, trp5-48, can1-100*
A 3-3:	a; *ade2-1, lys1-1, trp5-48*
A 14-2B:	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, met10-4, ura4-11*
A 14-33 A 14-76 A 176-1A	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14*
A 14-137 A 14-21	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, met10-4, leu2, ura4-11*
A 14-11D A 50-12A A 50-13A	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, leu2, ura4-11*
A 2-1A A 14-2 A 14-136	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, leu2*
A 4-3B:	α; *SUP6-1, trp1, his2, cdc14, met10-4 (arg4-0, trp5-48, lys1-1)?*
A 5-1:	*SUP6-1, cdc14, his2, met10-4 (trp5-48, lys1-1, arg4-0)?*
A 28-1B A 14-28	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, ura4-11*
A 14-69:	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, met10-4, leu2*
A 14-44:	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, his2, cdc14, leu2*
A 14-11B:	*ade2-1, lys1-1, trp5-48, can1-100, met10-4*
A 14-93:	*ade2-1, lys1-1, trp5-48, can1-100, his2*
A 14-109:	*ade2-1, lys1-1, trp5-48, can1-100, his2, ura4-11*

[1] *leu2* only in strain A 3-1*

(Cont'd)

Table 2 (Cont'd)

A 20-x:	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1-x, his2, cdc14, leu2*
A 14-2A A 14-3C	*ade2-1, lys1-1, trp5-48, can1-100, leu2*
A 133-6C A 14-133 A 133-8C	*ade2-1, lys1-1, trp5-48, can1-100, cdc14, ura4-11*
A 14-1C:	*ade2-1, lys1-1, trp5-48, can1-100, leu2, ura4-11*
A 22-4C	*ade2-1, lys1-1, trp5-48, can1-100, met10-4, leu2*
A 14-15:	*ade2-1, lys1-1, trp5-48, can1-100, met10-4, leu2, ura4-11*
A 6-4C A 7-17C A 8-8A A 9-2B A 10-13B A 11-4B A 12-7D A 13-5A	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, met10-4, ura4-11*
A 819-18B:	*ade2-1, lys1-1, trp5-48, rad1-18*
A 818-4C:	*ade2-1, lys1-1, trp5-48, can1-100, rad1-18*
A 817-1C:	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, met10-4, rad1-18*
A 816-4A:	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, ura4-11, leu2-1, rad1-18*
A 816-4D:	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, met10-4, rad1-18*
A 019-2A:	*ade2-1, lys1-1, trp5-48, can1-100, rad1-5*
A 018-2D:	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, ura4-11, rad1-5*
A 017-8A:	*ade2-1, lys1-1, trp5-48, can1-100, met10-4, ura4-11, rad1-5*
A 016-13B:	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, leu2, ura4-11, rad1-5?*
A 016-4A:	*ade2-1, lys1-1, trp5-48, can1-100, met10-4, rad1-5?*

(Cont'd)

Table 2 (Cont'd)

A 029-8B:	*ade2-1, lys1-1, trp5-48, can1-100, rad2-1*
A 028-8D:	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, met10-4, rad2-1*
A 027-4D A 027-7B	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, rad2-6*
A 027-1D A 027-7D	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, met10-4, leu2, ura4-11, rad2-6*
A 039-2B:	*ade2-1, lys1-1, can1-100, rad3-2*
A 038-2B:	*ade2-1, lys1-1, trp5-48, can1-100, rad3-2*
A 037-5B	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, leu2, rad3-2*
A 036-6A:	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, leu2, rad3-2*
A 036-13C A 036-6C	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, met10-4, ura4-11, rad3-2* .
A 049-18D:	*ade2-1, lys1-1, trp5-48, can1-100, rad4-4*
A 048-1D:	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, leu2, rad4-4*
A 047-1D:	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, leu2, rad4-4*
A 046-5A:	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, met10-4, ura4-11, leu2, rad4-4*
A 046-1B:	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, rad4-4*
A 046-4B:	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, leu2, ura4-11, rad4-4*
A 046-17C	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, met10-4, rad4-4*

(Cont'd)

Table 2 (Cont'd)

A 169-26C:	*ade2-1, trp5-48, lys1-1, can1-100, rad16-1*
A 168-5D:	*ade2-1, trp5-48, lys1-1, can1-100, his2, cdc14, leu2, rad16-1*
A 167-28B:	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, met10-4, rad16-1*
A 167-29A:	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, leu2, ura4-11, rad16-1*
A 189-3B **A 188-1B**	*ade2-1, lys1-1,* (*trp5-48?*), *rad18-1*
A 187-3B:	*ade2-1, lys1-1, trp5-48 , can1-100, his2, cdc14, rad18-1*
A 186-13D **A 186-3A**	*ade2-1, lys1-1, trp5-48, can1-100, his2, cdc14, leu2, ura4-11, rad18-1*
A 186-3B:	*ade2-1, lys1-1, trp5-48, can1-100, SUP6-1, met10-4, rad18-1*

of the red color in adenine-requiring strains.

Plates on which the dissections were performed were allowed to grow at 25° then replica plated directly onto the appropriate media to test for auxotrophic requirements and temperature sensitivity (YEPD incubated at 36°). Tetrads with conversion were generally picked and retested to confirm their phenotypes. When sectors of red color indicated post-meiotic segregation for *SUP6-1*, both parts of the sector were picked and retested. Spores were classified as having undergone postmeiotic segregation only if all unlinked alleles were identical in the two segments. Because of growth differences between strains with and without suppressors, that is, suppressor-carrying strains grew more slowly on YEPD but were at an advantage on defined medium, sectors were not required to be equal in size to indicate postmeiotic segregation. However, any sector less than one-eighth the size of the colony was ignored.

Mating and Sporulation for Random Spore Analysis

Haploid strains with independently isolated secondary mutations of *SUP6-1* were grown to log phase in liquid YEPD on a shaker at 25°. The two parents were mixed and left shaking for several hours, until they reached late log or early stationary phase. These mated cultures were washed and resuspended in 1% potassium acetate. They were kept shaking vigorously for four days or more before processing.

Preparation of Spore Suspension

Sporulated cultures were washed and resuspended in .3 ml .5M Tris (pH 8.8) plus .05 ml undiluted glusulase. The mixture was incubated for a minimum of one hour at 37°. This suspension was washed and resuspended in 5ml water. A French Pressure Cell (Aminco, rapid-fill and manual-fill models) was sterilized either by passing steam through the cylinder for 10 minutes and allowing to cool or by leaving a 1% Roccal solution in the cell for 20—30 minutes, then flushing with sterile distilled water. The suspension was put into the cell, then collected by drops while a pressure of 15,000 PSI was maintained. If by haemocytometer count there remained greater than 1% vegetative cells or groups of spores, the suspension was passed through the pressure cell repeatedly until a count of 99% single spores was achieved.

Selection of Meiotic Recombinants and Calculation of Recombination Frequencies

The samples prepared by the technique described above were plated on complete defined medium and on -ALT triple omission medium to select for colonies with a functional suppressor. Plates were incubated at 25° for seven days before counting. These prototrophs were picked and tested for the markers flanking *SUP6*, that is histidine and methionine requirements and temperature sensitivity (growth on YEPD at 36°).

The prototroph frequencies given in Table 3 represent the mean of the frequencies obtained in repeats of each cross. For each run, colony numbers on all plates were totalled, unless the plate count exceeded 1000. Therefore, the individual recombination values are a weighted average, given as the number of prototrophs per 10^6 spores.

Gamma Ray Irradiation and Mapping

The parental strains were mixed on YEPD, allowed to mate for several hours, then streaked on -leucine, uracil double omission medium to select for diploid clones. These were picked after three days' growth at 25°, transferred to liquid YEPD, and kept shaking until they reached the desired concentration,overnight for log phase cultures, 48 hours for stationary phase. Cultures were washed, plated on -lysine, tryptophan omission medium and irradiated with a ^{60}cobalt source delivering approximately 3 krad/minute to a total dose of 3, 6, 9 and 12 krad. Plates were incubated for seven days at 25° before counting. Controls for survival were plated on a complete defined medium; the doses used were sub-lethal.

Testing Sensitivity to Ultraviolet Light

UV sensitivity was tested in the dissected spores of crosses involving one of the *rad*$^-$ alleles by replica plating from the plate on which the dissection was performed to YEPD, and exposing this to ultraviolet irradiation. In the case of less sensitive alleles or doubtful readings, a "spot test" was performed. The culture to be tested was suspended in water at a concentration of 5 x 10^6 cells/ml and small drops of these suspensions were put onto YEPD plates. With either technique, the plates were exposed to a dose of ultraviolet light which resulted in a clear distinction between sensitive and resistant colonies, from approximately 500 to 1000 ergs/mm^2. The dose rate was 14 ergs/mm^2/sec as measured with a Latarjet dosimeter.

RESULTS

I. Random Spore Analysis and Mapping

Secondary mutations of *SUP6-1* were selected in strain A14-2B. Although selected on plates with a higher concentration of canavanine sulfate than that normally used to score canavanine sensitivity (200 mg/1 rather than 60 mg/1), a variety of intermediate phenotypes for the degree of suppression were recovered. Total loss of suppression made a strain phenotypically adenine, lysine and tryptophan requiring and canavanine resistant. Weak suppressors generally suppressed *trp5-48* and *lys1-1*, but *ade2-1* remained partially or fully expressed. The selection procedure made it unlikely that all ranges of suppression would have been recovered.

Only strains with complete loss of suppression were used for further study. Eleven such strains were tested. All showed the loss to be in *SUP6* and not in a secondary modifying locus. One mapped in the same position as the original *SUP6-1* mutation; the others are diagrammed in Figure 2.

Prototroph Frequencies and Genotypes of Flanking Markers

The results of all pairwise crosses of strains carrying one of ten secondary mutations of *SUP6-1* or the wild-type allele at that locus are presented in Table 3. The secondary mutations were in haploid derivatives of strain A20, and the wild-type allele came from strains A2-1A and A22-4C. This table shows the prototroph frequencies and the distribution of outside markers among selected prototrophic meiotic products. Given the diploid $\frac{A\ 1+\ B}{a\ +2\ b}$,

R_1 is the more common recombinant class among selected intragenic recombinants, expected from a single crossover between the alleles or a conversion with an adjacent crossover. a ++ B.

R_2 is the rare recombinant class. Seen as a triple exchange, it requires conversion of one allele with crossing-over removed from the site of conversion. A ++ b.

P_1 is the parental combination of outside markers which entered the cross with the proximal allele. A ++ B.

P_2 is the parental combination of outside markers which entered the cross with the distal allele. a ++ b.

The proximal or distal position of an allele and thus P_1 and P_2 were determined by the order suggested by the R_1:R_2 inequality.

Control for Reversion and Second Site Suppressor Formation

The effect of reversion and new suppressor formation on prototroph production in heteroallelic crosses can be estimated from the results of the homoallelic (self) crosses. The mean frequency of prototrophs in these crosses was 14.4×10^{-6} with a standard deviation of 18.7×10^{-6}. Since recombination between alleles *1-8*, *1-10*, and *1-11* ranged from 13 to 70 prototrophs per 10^6 spores, these three alleles are either independently isolated alleles at the same position, or are too close to allow resolution from the background of mutation occurring at meiosis. A second mapping procedure, using the inequality of the two recombinant classes, placed these three alleles adjacent to one another. With the exception of cross *1-8* x *1-3*, they behaved similarly in all crosses. The characteristics of allele *1-2* in two-point crosses can most simply be explained

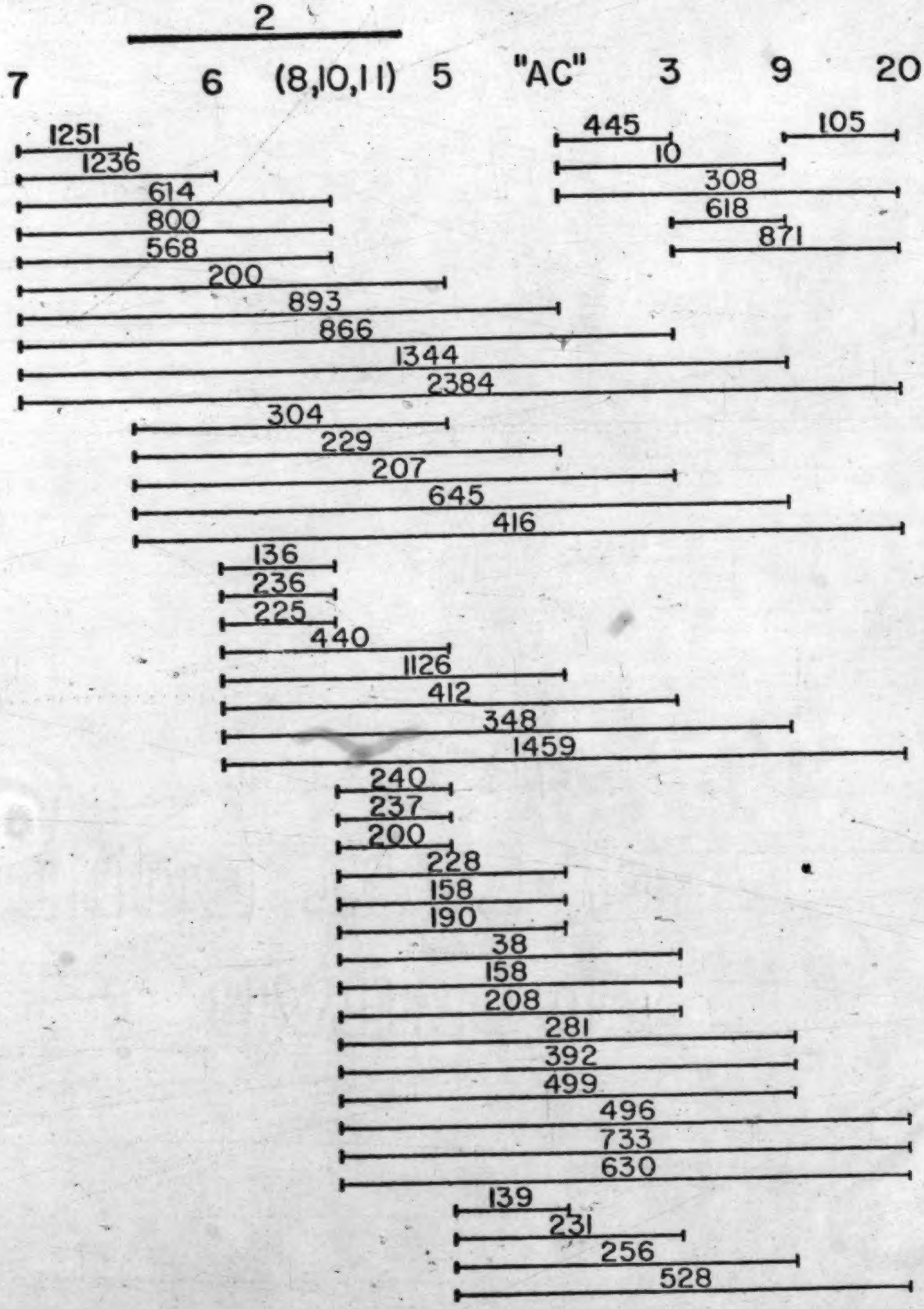

2
7
6
(8,10,11)
5
"AC"
3
9
20
1251
1236
614
800
568
200
893
866
1344
2384
304
229
207
645
416
136
236
225
440
1126
412
348
1459
240
237
200
228
158
190
38
158
208
281
392
499
496
733
630
139
231
256
528
445
10
308
618
871
105

Fig. 2. Meiotic fine structure map of *SUP6*. Prototroph frequencies/10^6 spores.

TABLE 3

Meiotic recombination frequencies and distribution of outside markers among recombinants

Alleles	Prototroph frequency x 10^6	Number of repeats	Range of values	P_1	P_2	R_1	R_2
1-7 x *1-7*	4	1		35	26	5	4
1-7 x *1-2*	1251	2	1150-1350	157	140	143	86
1-7 x *1-6*	1236	5	867-1715	335	335	305	194
1-7 x *1-8*	614	1		100	79	93	34
1-7 x *1-10*	800	1		102	92	66	29
1-7 x *1-11*	568	1		131	111	92	47
1-7 x *1-5*	200	4	115-305	201	283	189	69
1-7 x *AC*	893	5	631-1281	225	141	191	42
1-7 x *1-3*	866	2	852-880	134	128	169	61
1-7 x *1-9*	1344	2	1034-1654	207	146	187	54
1-7 x *1-20*	2384	1		80	81	109	28
1-2 x *1-2*	12	1		90	102	33	43
1-2 x *1-6*	71	2	54-89	165	154	90	63
1-2 x *1-8*	9	2	7-11	15	20	4	4
1-2 x *1-10*	29	1		56	88	64	30
1-2 x *1-11*	26	2	26-27	82	102	77	22
1-2 x *1-5*	304	2	241-368	157	180	141	55
1-2 x *AC*	229	6	180-320	217	153	163	60
1-2 x *1-3*	207	2	175-239	108	140	97	84
1-2 x *1-9*	645	2	497-793	169	216	141	71
1-2 x *1-20*	416	1		67	109	88	23
1-6 x *1-6*	14	1		45	71	19	17
1-6 x *1-8*	136	1		98	94	56	44
1-6 x *1-10*	236	1		64	67	55	30
1-6 x *1-11*	225	1		98	85	73	38
1-6 x *1-5*	440	2	380-501	199	186	154	54
1-6 x *AC*	1126	2	865-1386	205	136	159	52

(Cont'd)

Table 3 (Cont'd)

Alleles	Prototroph frequency x 10^6	Number of repeats	Range of values	P_1	P_2	R_1	R_2
1-6 x *1-3*	412	2	369-456	162	184	171	65
1-6 x *1-9*	348	1		101	77	90	25
1-6 x *1-20*	1459	2	1059-1858	186	156	190	60
1-8 x *1-8*	18	1		8	12	2	3
1-8 x *1-10*	31	2	15-48	57	44	28	15
1-8 x *1-11*	13	1		7	6	6	0
1-8 x *1-5*	240	1		87	108	50	40
1-8 x *AC*	228	4	193-298	143	123	136	55
1-8 x *1-3*	38	4	19-80	39	53	34	18
1-8 x *1-9*	281	1		84	87	90	34
1-8 x *1-20*	496	3	168-696	210	282	231	69
1-10 x *1-10*	21	2	13-29	18	12	3	2
1-10 x *1-11*	70	2	40-101	48	51	15	13
1-10 x *1-5*	237	6	155-459	223	371	242	125
1-10 x *1-AC*	158	3	140-169	156	165	146	58
1-10 x *1-3*	158	1		82	93	73	41
1-10 x *1-9*	392	1		70	102	96	30
1-10 x *1-20*	733	2	731-736	82	106	94	17
1-11 x *1-11*	6	2	1-11	7	10	2	2
1-11 x *1-5*	200	1		53	95	45	28
1-11 x *AC*	190	3	114-233	185	158	172	62
1-11 x *1-3*	208	1		71	100	52	37
1-11 x *1-9*	499	3	167-716	139	186	108	63
1-11 x *1-20*	630	1		81	110	90	17
1-5 x *1-5*	11	1		17	23	4	14
1-5 x *AC*	139	3	88-182	162	134	117	57
1-5 x *1-3*	231	2	118-343	107	176	95	88
1-5 x *1-9*	256	1		89	90	87	27
1-5 x *1-20*	528	1		84	110	78	22

(Cont'd)

Table 3 (Cont'd)

Alleles	Prototroph frequency x 10^6	Number of repeats	Range of values	P_1	P_2	R_1	R_2
AC x *AC*	2	2	1-3	7	15	3	0
AC x *1-3*	445	4	238-692	43	111	51	48
AC x *1-9*	10	4	4-18	61	62	63	14
AC x *1-20*	308	1		95	85	90	23
1-3 x *1-3*	67	1		102	113	26	40
1-3 x *1-9*	618	1		62	26	39	17
1-3 x *1-20*	871	1		104	55	72	28
1-9 x *1-9*	0	1		3	5	1	0
1-9 x *1-20*	105	1		84	80	98	34
1-20 x *1-20*	3	1		32	28	7	10

if this marker is a deletion covering *1-6*, *1-8*, *1-10*, and *1-11*.

Although the two crosses *1-8* x *1-3* and *AC* x *1-9* showed consistently low prototroph frequencies averaging 38 and 10 x 10^{-6} respectively, these alleles are believed to be at different sites, since mapping by the segregation of outside markers did not place them adjacent to each other. With the exception of these two crosses and the alleles discussed above, heteroallelic crosses gave recombination frequencies at least an order of magnitude higher than self crosses.

A second control for the appearance of new suppressors involved crossing a sample of the presumed meiotic recombinants to strains without a suppressor and with complementary outside markers. The purpose of these crosses was to test whether the selected suppressors were at the *SUP6* locus, that is, still linked to *cdc14* and *met10*. Five prototrophic meiotic products were picked from each of ten different heteroallelic crosses and approximately sixteen asci dissected from each cross. Only one selected prototroph had a suppressor at a locus other than *SUP6*. Eight prototrophs were picked from two different homoallelic crosses, and of these, six carried suppressors not closely linked to *cdc14* and *met10*. The one second-site suppressor from the heteroallelic cross and the six from homoallelic crosses all showed loose linkage with *cdc14* and *met10*, which suggests that the new suppressor in all cases was *SUP11*. *SUP11* is proximal to *SUP6*, about 26 map units from *cdc14* and 32 from *met10* (Mortimer and Hawthorne 1973). Those suppressors scored as *SUP11* showed recombination frequencies ranging from 21% to 30% with *cdc14* and 24% to 34% with *met10*.

This set of dissections from self-crosses provided a simple explanation for the observation that although allele *1-2* behaved like a deletion in recombination studies, it showed a nearly average rate of suppressor formation in self crosses (12×10^{-6}). The majority of suppressors selected in self crosses were forward mutations to suppression at a second locus and not reversions of the secondary *SUP6-1* mutation.

The following experiment showed that most of the reversion to suppression arose after transfer to sporulation medium. Twenty-five crosses were set up as for random spore analysis but at the time when they would normally have been transferred to sporulation medium, they were plated on complete defined and -ALT media. The prototroph frequencies ranged from 0.07×10^{-6} to 0.45×10^{-6}. Five self crosses showed prototroph frequencies as high as those found in heteroallelic crosses. Therefore, mutation and not recombination is believed to have been largely responsible for the appearance of suppressors during diploid mitotic growth.

Prototroph Frequencies in an Unselected Sample

There existed the possibility that the -ALT medium on which prototrophs were selected did not always allow for the expression of a functional suppressor generated during meiosis, and that those prototrophs which grew on -ALT were only a sample of the recombinants produced. To clarify this, three crosses in which the prototroph frequency was high, *1-7* x *AC*, *1-9* x *1-2*, and *1-6* x *AC* were sporulated and the spore suspension was prepared as usual, but spores were plated on YEPD and complete defined medium rather than on -ALT. These were replica plated to -ALT or -LT medium. Colonies grown on the complete defined medium showed a high frequency of small sectors with the suppressor phenotype, presumably suppressor mutations. The limiting supply of lysine on the defined medium may have been responsible for selection of suppressors during growth. On YEPD the results were clearer, and they are presented in Table 4.

Although the estimate of prototroph frequency in the unselected sample was based on a very small number, the rough correspondence between prototroph frequencies obtained from selective and non-selective experiments makes it unlikely that only a fraction of suppressor-carrying spores were able to grow on -ALT medium.

Lack of Polarity as Revealed by the Parental Classes of Prototrophs

From columns 5 and 6 of Table 3 and column 2 of Table 5, we see that there was no consistent inequality between P_1 and P_2. If the same parental class were greater in all crosses, this would indicate a gradient of conversion from one end of the locus to the other. Approximately 40% of the crosses between alleles at different sites showed a significant inequality of P_1 and P_2, but there was no consistent pattern to this inequality, nor was it characteristic of crosses involving any one particular allele.

Inequality of Parental and Recombinant Classes of Outside Markers

Column 4 of Table 5 shows the fraction of selected prototrophs in crosses between alleles at different sites which were recombinant for the flanking markers *cdc14* and *met10*. The percent recombination for flanking markers among intragenic recombinants at *SUP6* varied from 32%

TABLE 4

Frequency of prototrophs on non-selective medium

		Prototrophs			Frequency of prototrophs x 10^6		
Cross	Number of colonies on YEPD	whole colonies	mixed colonies	colonies with small sectors	whole colonies	whole + mixed colonies	Frequency of prototrophs x 10^6 in selected experiments (see Table 3)
1-7 x *AC*	1786	1	1	1	560	1120	893
1-6 x *AC*	1577	1	1	0	634	1268	1126
1-9 x *1-2*	3998	3	2	1	750	1250	645

TABLE 5

Fraction P /P, R /R and R/Total for crosses involving alleles which map at different sites

	P_1/P_1+P_2	R_1/R_1+R_2	R_1+R_2/Total
1-7 x *1-2*	.529	.624	.435
1-7 x *1-6*	.500	.611	.427
1-7 x *1-8*	.559	.732	.415
1-7 x *1-10*	.526	.695	.329
1-7 x *1-11*	.541	.662	.365
1-7 x *1-5*	.415 *	.733	.348
1-7 x *AC*	.615 *	.820	.389
1-7 x *1-3*	.511	.735	.467 +
1-7 x *1-9*	.586 *	.776	.406
1-7 x *1-20*	.497	.796	.460 +
1-2 x *1-5*	.466	.719	.368
1-2 x *AC*	.586 *	.731	.376
1-2 x *1-3*	.435 *	.536 +	.422
1-2 x *1-9*	.439 *	.665	.355
1-2 x *1-20*	.381 *	.793	.387
1-6 x *1-8*	.510	.560 +	.342
1-6 x *1-10*	.489	.647	.394
1-6 x *1-11*	.536	.658	.378
1-6 x *1-5*	.517	.740	.351
1-6 x *AC*	.601 *	.754	.382
1-6 x *1-3*	.468	.725	.405
1-6 x *1-9*	.567	.783	.392
1-6 x *1-20*	.544	.760	.422
1-8 x *1-5*	.446	.556 +	.316
1-8 x *AC*	.538	.712	.418
1-8 x *1-3*	.424	.654	.361
1-8 x *1-9*	.491	.726	.420
1-8 x *1-20*	.427 *	.770	.379

(Cont'd)

Table 5 (Cont'd)

	P_1/P_1+P_2	R_1/R_1+R_2	R_1+R_2/Total
1-10 x *1-5*	.375 *	.659	.382
1-10 x *AC*	.486	.716	.389
1-10 x *1-3*	.469	.640	.394
1-10 x *1-9*	.686 *	.762	.423
1-10 x *1-20*	.436	.847	.371
1-11 x *1-5*	.358	.616	.330
1-11 x *AC*	.539	.735	.406
1-11 x *1-3*	.415 *	.584 +	.342
1-11 x *1-9*	.428 *	.632	.345
1-11 x *1-20*	.424 *	.841	.359
1-5 x *AC*	.547	.672	.370
1-5 x *1-3*	.378 *	.519 +	.393
1-5 x *1-9*	.497	.763	.389
1-5 x *1-20*	.433	.780	.340
AC x *1-3*	.279 *	.515 +	.391
AC x *1-9*	.496	.818	.385
AC x *1-20*	.528	.796	.386
1-3 x *1-9*	.705 *	.696	.389
1-3 x *1-20*	.654 *	.720	.386
1-9 x *1-20*	.512	.742	.446 +
Mean	.496	.703	.386

*Significantly different from 50% at 5% level.

+Not significantly different from 50% at 5% level.

to 47%. In all but three crosses, the inequality was significant at the 5% level as determined by a goodness-of-fit chi-square.

Table 6 shows that the excess of parental over recombinant genotypes was not a result of selection for a particular phenotype with respect to outside markers. When the phenotypes of the four possible classes were rearranged by changing the original parental combinations, the asymmetry was maintained.

Since some allelic crosses were made more than once with outside markers in different configurations, calculations of the amount of variation in the parental to recombinant ratio between crosses involving different alleles required pooling the data for crosses performed with the same two *SUP6* alleles. The amount of variation between different allelic crosses was highly significant, as determined by chi-square analysis of a 2 x 48 contingency table of heteroallelic crosses ($\chi^2 = 91$; $p < .001$). The 23 allelic crosses repeated with outside markers in different configurations were also tested for within-cross heterogeneity. Analysis of contingency tables made up of crosses involving the same *SUP6* alleles with different flanking marker arrangements showed this within-cross heterogeneity to be significant at the 5% level ($\chi^2_{29} = 44.15$; $p = .05-.025$). Although there were significant differences in the parental to recombinant ratio for crosses involving the same *SUP6* alleles as well as for crosses between different alleles, the variation was far greater between crosses involving different alleles. This suggests that there was an allele- or perhaps position-specific effect in the frequency of outside marker exchange accompanying intragenic recombination.

TABLE 6

Distribution of flanking markers among recombinants from crosses with different arrangements of outside markers.

Cross 1: $\dfrac{+ \quad \mathit{1}+ \quad \mathit{met10}}{\mathit{cdc14} \quad +\mathit{2} \quad +}$ Cross 2: $\dfrac{\mathit{cdc14} \quad \mathit{1}+ \quad +}{+ \quad +\mathit{2} \quad \mathit{met10}}$

Cross 3: $\dfrac{\mathit{cdc14} \quad \mathit{1}+ \quad \mathit{met10}}{+ \quad +\mathit{2} \quad +}$ Cross 4: $\dfrac{+ \quad \mathit{1}+ \quad +}{\mathit{cdc14} \quad +\mathit{2} \quad \mathit{met10}}$

Alleles of *SUP6*	Cross	Fraction					Number tested
		P_1	P_2	R_1	R_2	R	
1-7 x *1-6*	1	.29	.30	.24	.17	.41	296
	2	.28	.31	.26	.15	.41	289
	3	.30	.29	.25	.16	.41	297
	4	.27	.25	.30	.19	.49	287
1-7 x *1-5*	1	.39	.33	.23	.05	.28	57
	2	.37	.33	.23	.07	.30	112
	3	.24	.39	.28	.09	.37	287
	4	.26	.39	.24	.11	.35	286
1-10 x *1-5*	1	.26	.38	.21	.05	.36	287
	2	.18	.39	.34	.09	.43	265
	3	.26	.30	.21	.13	.34	95
	4	.25	.39	.22	.15	.37	314

The proportion of prototrophs with flanking markers recombined was positively correlated with the prototroph frequency ($r=.43$; $df=46$; $p<.005$), yet there was no correlation between the proportion of recombinants and the number of intervals between the alleles (that is, the number of spaces between allele positions taken from the map in Figure 2). Because the number of intervals between alleles is thought to be a better measure of distance than prototroph frequency in this system, changes in the parental to recombinant ratio appear to have been related to those factors which caused perturbations in the recombination frequency rather than to the distance between the alleles. No other patterns in P versus R were revealed. For example, no particular allele showed a tendency for higher or lower flanking marker recombination in most crosses, nor were there any characteristics to distinguish crosses in the distal from those in the proximal section of the locus.

Mapping from the $R_1 : R_2$ Inequality

The inequality of the two classes of prototrophs recombinant for outside markers was used to generate the map shown in Figure 2. As seen in Table 5, in all but six crosses of alleles at different sites, the difference between R_1 and R_2 was significant at the 5% level. There were no internal inconsistencies when all pairs of alleles were ordered by this method. From 52% to 85% of the recombinants were of the more common recombinant class. Out of the 23 crosses in which the same two alleles were crossed with reciprocal outside marker configurations, only one did not show the expected reversal of genotype of the two recombinant classes. However, in neither cross was one recombinant class significantly greater than the other. The amount of variation in R_1:R_2 for different allelic crosses was highly significant ($\chi^2_{47}=261$; $p<<.001$). The variation between crosses performed with the same pair of alleles but different flanking marker configurations was also significant (χ^2_{29}=90.14; p<.001). This value is the sum of the within-cross variations.

Patterns of R /R and P /P Ratios

On the basis of models of recombination which involve hybrid DNA formation leading to conversion and associated reciprocal recombination, one expects only R_1 recombinants from a length of hybrid DNA which ends between the alleles and is resolved as a crossover. Therefore, the greater the proportion of prototrophs which arise from hybrid endings, the greater the inequality of the two recombinant classes. Hybrid DNA endings may also generate an inequality of the two parental classes. Non-crossover hybrid which covers only the proximal allele will give a P_1 product and non-crossover hybrid which covers just the distal allele will give P_2. Therefore, if hybrid DNA formation is initiated at the proximal end of the gene and migrates into the gene, but often covers only one allele, P_1 will exceed P_2. If hybrid formation is initiated distally, then P_2 is expected to be greater than P_1.

Figure 3 shows the relationship of R_1/R to P_1/P for all heteroallelic combinations. The expectation that as R_1/R increases, the difference between P_1 and P_2 should increase as well, was not fulfilled. However, the points are not arranged randomly, and several allele-specific characteristics emerged. The most notable were the high R_1/R values for crosses involving allele *1-20* and the low values for most crosses of allele *1-3*.

One might also expect changes in R_1/R and P_1/P to be accompanied by changes in the reliability of prototroph frequency as a measure of

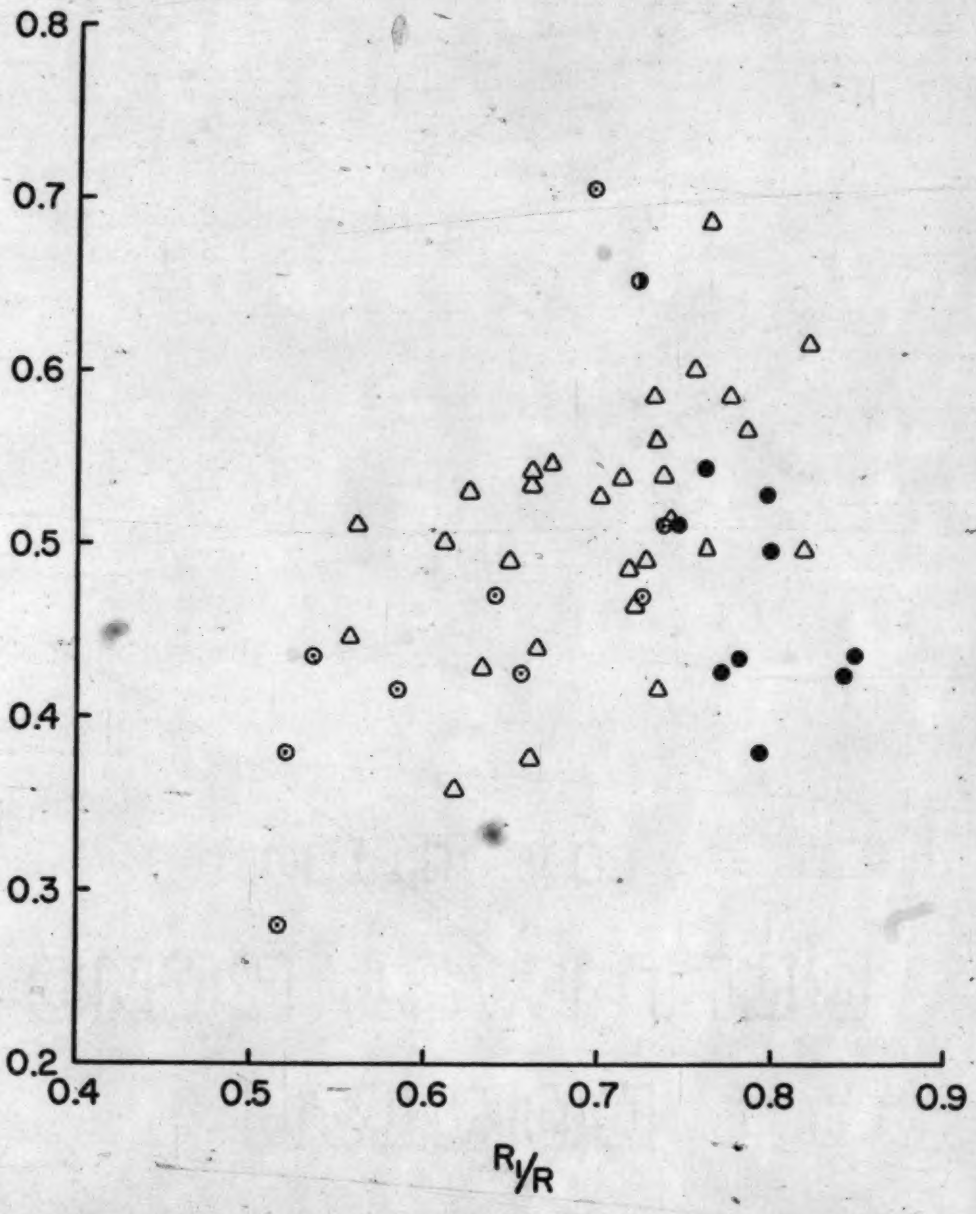


Fig. 3. Relationship between R_1/R and P_1/P for the 48 heteroallelic crosses.

●	crosses involving allele *1-20*
⊙	crosses involving allele *1-3*
◐	*1-3* x *1-20*
△	all other crosses

distance between alleles. Since the most clearly recognized marker effects are those which appear to operate on the level of correction (Kitani and Olive 1967, 1970; Leblon 1972a,b; Leblon and Rossignol 1973; Kitani and Whitehouse 1974b), one might obtain a more accurate map using those crosses in which recombination was primarily due to hybrid endings. However, selection of crosses which showed a significant difference between P_1 and P_2, or an R_1/R ratio greater than .7 did not change the non-additive quality of the map. The principal coordinate analysis performed on the whole set of results cannot be done without a symmetrical matrix of recombination values, so a quantitative analysis of selected crosses was not possible.

Estimating a Linear Map and its Fit

The meiotic prototroph frequencies from two-point crosses are shown in Table 3 and in Figure 2. The frequencies did not appear to be related to the order based on flanking marker distribution, nor could they easily be used to arrange the alleles in any other sequence. However, an attempt was made to construct a map using prototroph frequencies alone. The technique of principal coordinate analysis was applied to this problem by Kenneth Morgan who also performed the analysis and provided the description which follows.

In order to obtain an initial guess of a one-dimensional map which would sufficiently represent the linear order of markers, the matrix of recombination data was treated as a Euclidian distance matrix and subjected to principal coordinate analysis (Gower 1966). Then, the fit of the obtained order to a linear map was assessed by reference to an empirical distribution generated by random sampling of permutations of the 11 markers. Brief accounts of these methods follow.

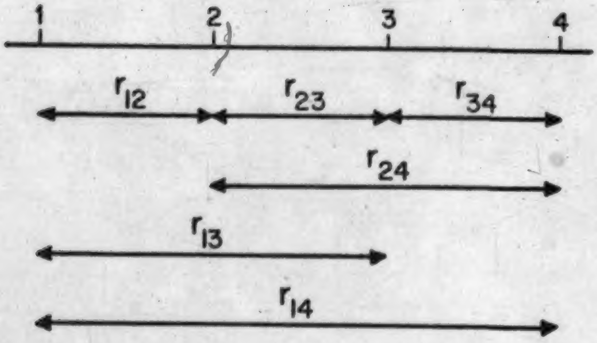
The matrix of pairwise recombination values was scaled by dividing by the largest observed value. Treating this matrix as a matrix of pairwise distances between markers, we obtain a centroid adjustment of the matrix and then extract eigenroots and eigenvectors (Morrison 1967). Unfortunately, some of the roots of the centroid-adjusted distance matrix were negative, so that the "distances" did not fit in a Euclidian space of (n-1) dimensions or less. Nevertheless, the dimension corresponding to the eigenvector associated with the largest positive eigenvalue was taken as a provisional linear map. This root was also the largest in absolute value. Coordinates for the markers were obtained as projections on this first dimension by multiplying the eigenvector by the square root of its associated eigenvalue.

We define a statistic of discrepancy from linearity for a given order of markers in the following way: for each span of markers (of three up to and including n markers) we subtract the sum of the recombination values of the adjacent intervals which are contained in the span from the recombination value for the two end markers which define the span and square the resulting difference. The values of all such comparisons are accumulated to a total measure of discrepancy from linearity for a given ordering of markers. Thus, the various ordered subsets of observed distances of adjacent intervals are compared with the observed recombination distances between the two markers which span an ordered subset. This method is diagrammed in Table 7.

To test the sufficiency of an arbitrary linear map by the value of its discrepancy statistic, an empirical distribution of the statistic

TABLE 7

Method used to obtain statistic of discrepancy.


1
2
3
4
r_{12}
r_{23}
r_{34}
r_{24}
r_{13}
r_{14}

$$D^2_{3(1)} = (r_{13} - [r_{12} + r_{23}])^2$$

$$D^2_{3(2)} = (r_{24} - [r_{23} + r_{34}])^2$$

$$D^2_{4(1)} = (r_{14} - [r_{12} + r_{23} + r_{34}])^2$$

ΣD^2 - Total statistic of discrepancy for this order was obtained for 200 approximately equally likely permutations of 11 markers utilizing the observed pairwise recombination values. Because of the symmetry of any given permutation and its inverse sequence, our empirical distribution determines the probability of discrepancy equal to or smaller than the smallest value of approximately 1%.

Figure 4 shows the statistic of discrepancy for some of the randomly generated sequences arranged in order of increasing nonlinearity, as well as for the outside marker map and the map generated by the analysis discussed above. These last two maps are quite similar. Alleles *1-5* and *1-3* were placed proximal to *1-8*, *1-10* and *1-11* by the matrix analysis; other alleles retained their same relative positions. The map generated by principal coordinate analysis showed less deviation from linearity than was shown by approximately 99% of random permutations of the 11 alleles, and the order derived from the R_1 - R_2 inequality fit a linear map better than 98% of random permutations.

Allele-Specific Effects on Prototroph Frequencies

Figure 5 represents an attempt to sort out consistent effects of certain alleles on recombination frequencies within *SUP6* using the R_1:R_2 map as a basis for interpretation. The number of intervals between alleles was taken as the number of spaces between allele positions. Alleles *1-8*, *1-10* and *1-11* have a common position. The number of intervals of any allele from allele *1-2* is the number of spaces from the closer end-point. All heteroallelic crosses are represented in this histogram of recombination frequency versus the number of intervals separating the two alleles. Dashed horizontal lines show the mean prototroph frequency for that number of intervals. Only when the number of intervals exceeded three did the recombination frequency increase with increasing separation of the alleles.

Randomly generated sequences

$D^2 \times 10^6$ ← 19.90 Order from principal coordinate analysis A

1. 26.95 ← 28.92 Order from $R_1 - R_2$ inequality B

2. 33.70

3. 35.90

4. 44.65

.

.

.

.

199. 709.84

200. 743.00

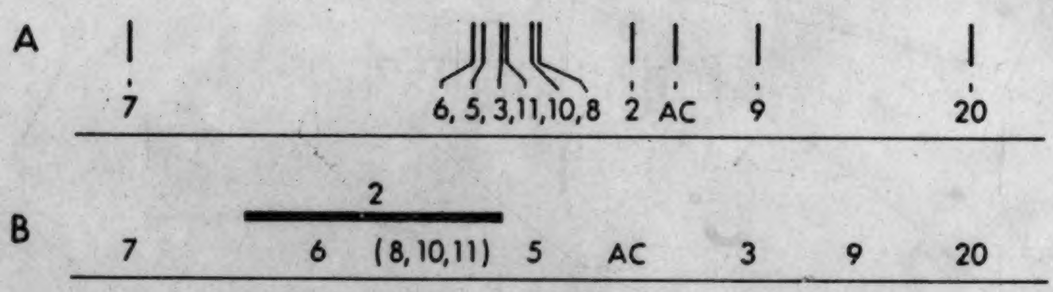

A
7
6, 5, 3,11,10,8
2
AC
9
20
B
2
7
6
(8,10,11)
5
AC
3
9
20

Fig. 4. Statistic of discrepancy for random permutations of the alleles, for the R_1 : R_2 map, and for the principal coordinate analysis map.

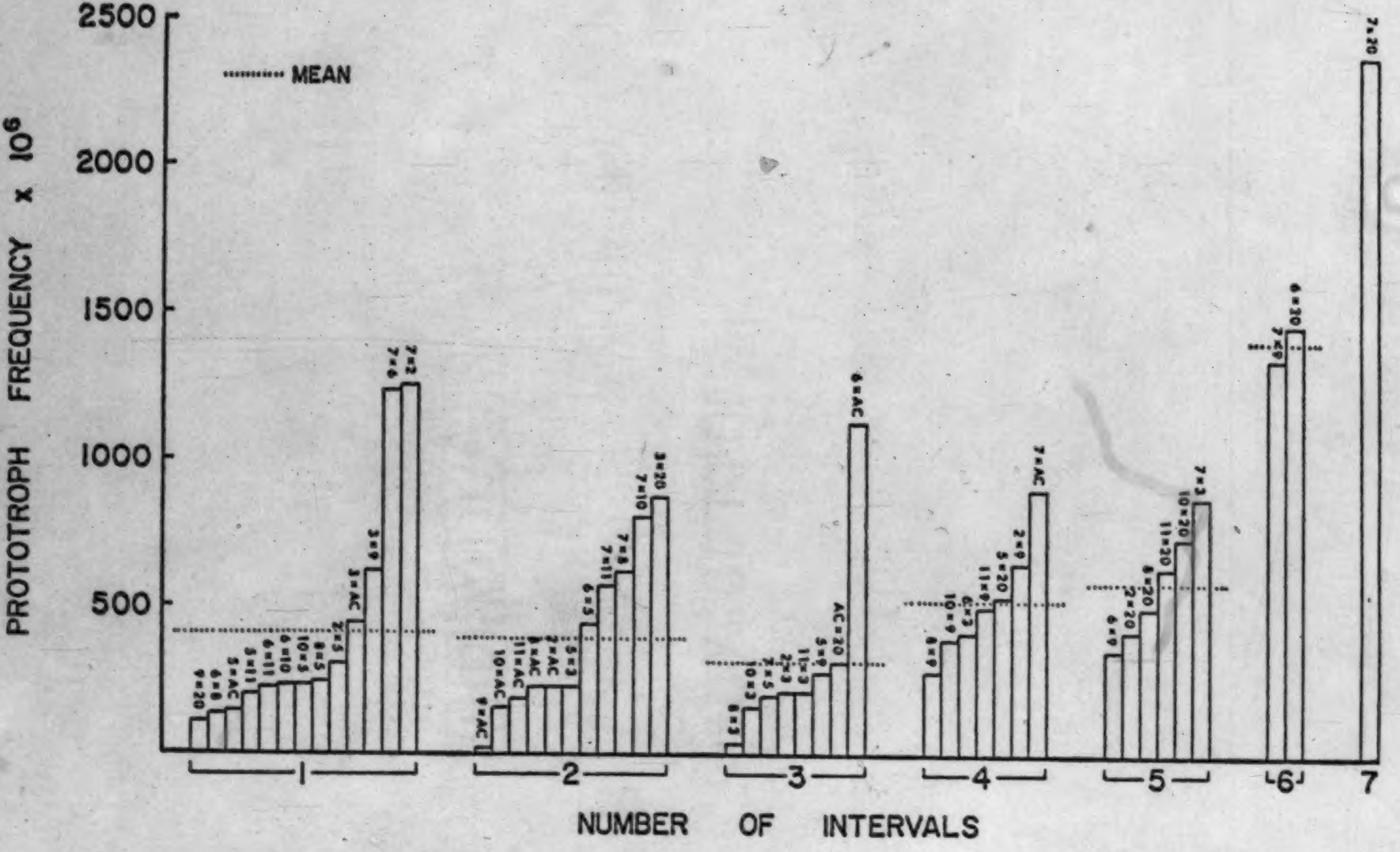


Fig. 5. Relationship between the number of intervals between the alleles and the prototroph frequency. Dotted lines represent the mean prototroph frequencies for crosses between alleles 1, 2, 3, 4, 5 and 6 intervals apart.

Crosses involving allele *1-7* (with the exception of *1-7* x *1-5*) stand out as having shown high recombination frequencies. Because allele *1-7* is on the proximal end of the map, the effect may have been one of position. The higher recombination frequencies could simply have been a function of the physical distance of *1-7* from the remainder of the alleles.

No other consistent allele-specific effects were obvious. However, certain combinations of alleles had unexpectedly high or low recombination frequencies not predicted from the behavior of the single mutants, most notably *1-6* x *AC* and *AC* x *1-9*.

Map Expansion

The extreme non-additivity of this map makes the detection of subtle patterns of deviations from linearity difficult. Nevertheless, an attempt was made to detect map expansion since, even in this system, expansion could reveal some of the characteristics of excision and hybrid endings. However, the standard plot of recombination frequency of a long interval (c) versus the map distance determined by adding the recombination frequencies of two intervening sub-intervals (a and b) revealed no tendency towards map expansion. Of 113 triplets tested, 42 showed a value of c/a+b greater than 1, indicative of expansion, 70 had a value less than 1, and one showed complete additivity (Fig. 6).

Because map expansion is sometimes seen to increase with the number of sub-intervals added (Mousseau 1967 discussed in Hastings 1975), longer distances were calculated using the sum of three or more shorter distances. This gave negative results as well. In addition, the number of intervals between two alleles was used as a measure of distance rather than the sum of the short distances. A plot of recombination frequency

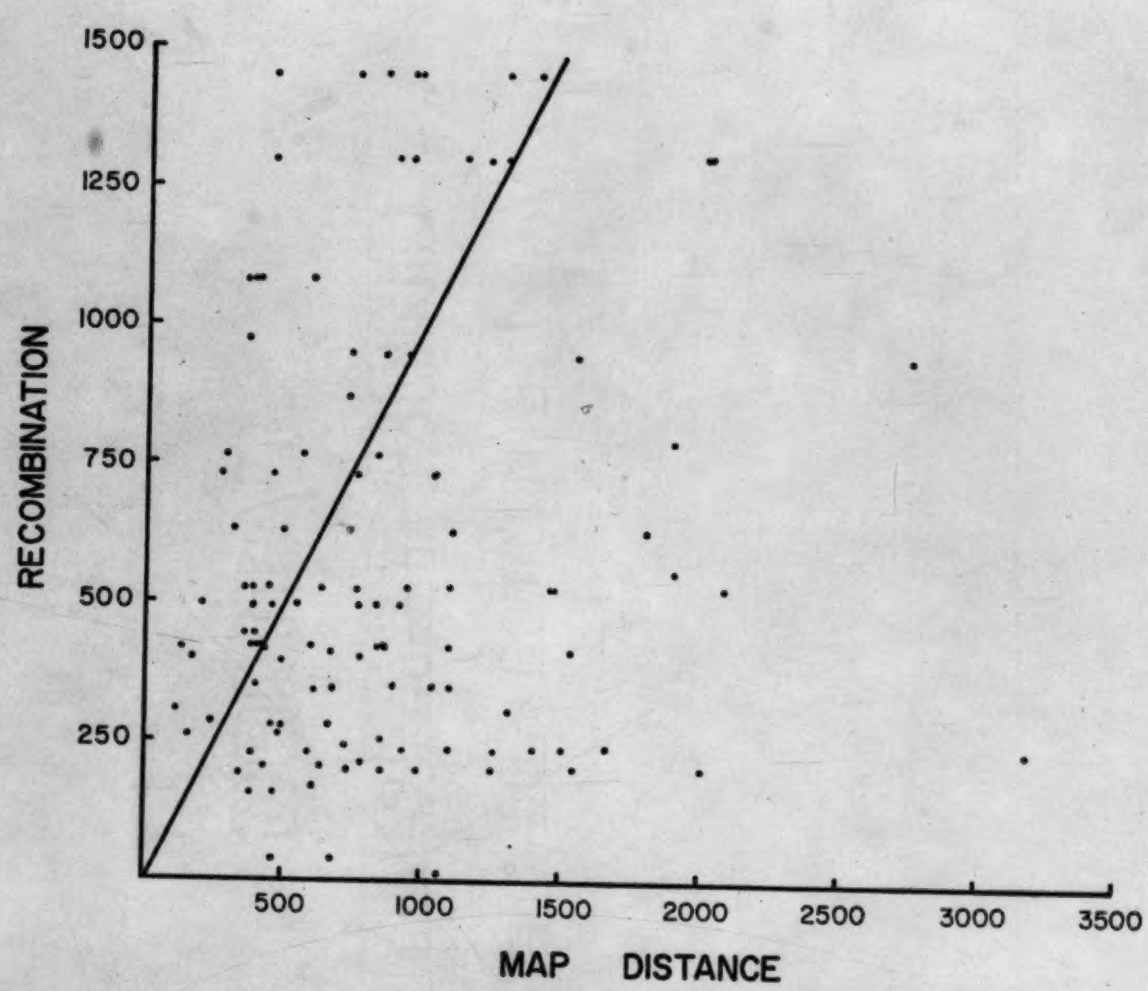


Fig. 6. Map expansion plot for two-point crosses of *SUP6* alleles. Recombination frequencies are compared with map distances computed by summing the recombination frequencies of two smaller intervals. Units are in prototrophs/10^6 spores. The line of slope 1 is drawn for reference.

per interval versus the number of intervals showed no increase in recombination frequency per unit distance with increasing distance between the two alleles.

II. Gamma Ray Induction of Mitotic Recombination

Rates of Prototroph Production

Another mapping procedure which made use of the frequency of gamma ray induced mitotic recombination in heteroallelic diploids was employed. This was a modification of the mapping technique of Manney and Mortimer (1964). Homoallelic diploids and strains with alleles believed to cover the same site showed low rates of prototroph formation, averaging 10.9 prototrophs/10^8 survivors/krad. Heteroallelic crosses were generally significantly higher, indicating that the majority of prototrophs generated in these diploids were recombinants. They displayed frequencies of prototroph production which increased linearly with dose, with correlation coefficients which averaged near 0.985. However, the rate of prototroph production for any one pair of alleles varied greatly in repeats of the same cross. This was true whether diploids were irradiated in log or in stationary phase. Variation between cultures irradiated in the same growth condition was as great as the differences between log and stationary phase. Table 8 shows the average rate of prototroph production for any pair of alleles as well as the individual values for each experiment. The underlined values were obtained from cultures irradiated in log phase. The remainder were from cultures grown for approximately 48 hours in liquid YEPD.

The average rates of prototroph formation induced by gamma rays were subjected to the same matrix analysis that was used for meiotic mapping.

TABLE 8

Rates of induction of prototrophs by gamma irradiation.

Alleles	Mean Prototrophs/10^8 survivor/Krad	Number of repeats	Prototrophs/10^8/Krad values from separate experiments
1-7 x *1-7*	9	1	9
1-7 x *1-2*	204	3	53, 199, 361
1-7 x *1-6*	154	2	143, 165
1-7 x *1-8*	161	3	38, 74, 370
1-7 x *1-10*	72	1	72
1-7 x *1-11*	183	3	106, 182, 262
1-7 x *1-5*	37	4	21, 27, 41, 59
1-7 x *AC*	117	2	115, 118
1-7 x *1-3*	54	13	10, 20, 23, 27, 27, 28, 32, 32, 41, 50, 58, 82, 87, 201
1-7 x *1-9*	183	3	73, 150, 327
1-7 x *1-20*	266	2	215, 316
1-2 x *1-2*	6	1	6
1-2 x *1-6*	15	3	14, 21,9
1-2 x *1-8*	8	3	5, 6, 13
1-2 x *1-10*	8	2	6, 9
1-2 x *1-11*	10	3	2, 9, 18
1-2 x *1-5*	38	3	23, 45, 47
1-2 x *AC*	36	1	36
1-2 x *1-3*	26	3	19, 28, 32
1-2 x *1-9*	149	4	81, 97, 147, 269
1-2 x *1-20*	56	3	46, 47, 75

(Cont'd)

TABLE 8 (Cont'd)

Alleles	Mean Prototrophs/10^8 survivor/Krad	Number of repeats	Prototrophs/10^8/Krad values from separate experiments
1-6 x *1-6*	6	1	6
1-6 x *1-8*	18	1	18
1-6 x *1-10*	29	1	29
1-6 x *1-11*	52	1	52
1-6 x *1-5*	41	2	28, 53
1-6 x *AC*	95	1	95
1-6 x *1-3*	67	2	40, 93
1-6 x *1-9*	243	2	161, 325
1-6 x *1-20*	140	1	140
1-8 x *1-8*	14	1	14
1-8 x *1-10*	8	1	8
1-8 x *1-11*	10	1	10
1-8 x *1-5*	53	1	53
1-8 x *AC*	59	1	59
1-8 x *1-3*	43	1	43
1-8 x *1-9*	120	1	120
1-8 x *1-20*	70	1	70
1-10 x *1-10*	8	1	8
1-10 x *1-11*	18	1	18
1-10 x *1-5*	80	1	80
1-10 x *AC*	74	1	74
1-10 x *1-3*	29	1	29
1-10 x *1-9*	74	1	74

(Cont'd)

Table 8 (Cont'd)

Alleles	Mean Prototrophs/10^8 survivor/Krad	Number of repeats	Prototrophs/10^8/Krad values from separate experiments
1-10 x *1-20*	109	1	109
1-11 x *1-11*	3	1	3
1-11 x *1-5*	232	1	232
1-11 x *AC*	54	1	54
1-11 x *1-3*	42	1	42
1-11 z *1-9*	79	1	79
1-11 x *1-20*	85	2	64, 105
1-5 x *1-5*	7	1	7
1-5 x *AC*	21	1	21
1-5 x *1-3*	43	2	46, 40
1-5 x *1-9*	72	2	63, 81
1-5 x *1-20*	151	1	151
AC x *AC*	24	1	24
AC x *1-3*	32	1	32
AC x *1-9*	13	1	13
AC x *1-20*	65	1	65
1-3 x *1-3*	3	1	3
1-3 x *1-9*	122	1	122
1-3 x *1-20*	119	1	119
1-9 x *1-9*	16	1	16
1-9 x *1-20*	79	1	79
*1-20*x *1-20*	16	1	16

The resulting map was not only non-additive, but it showed no obvious correlation with the maps generated either from meiotic prototroph frequencies or outside marker segregation in meiotic recombinants, with the exception of placing alleles *1-7* and *1-20* on opposite ends of the map.

Classification of events Induced by Gamma Rays

Despite the failure of the gamma irradiation technique to generate a map for this locus, some information was extracted from the sporulation and dissection of a sample of induced prototrophs. Table 9 shows the kinds of events responsible for prototroph formation after gamma irradiation. Included are all the analysed prototrophs which showed rearrangements in the region between *his2* and *met10*. All genotypes requiring more than one conversion, crossover, or conversion with an adjacent crossover in this region were classified as complex. Classes 4A, 6A, 8A, 11A, 1B and 4B were most likely the result of two events in this region; 5A, 10A and 7B required additional events. Diploids which fell into classes 3A, 1B and 4B showed high spore lethality after dissection.

The results of all dissections are summarized in Table 10. It may be seen that the proportions of different kinds of events differed for selected prototrophs of different colors. Table 11 shows the color distribution in a sample of crosses. The red color is caused by pigment accumulation in cells with a blocked adenine biosynthetic pathway. The color intensity can be a rough measure of the degree of suppression of the *ade2-1* mutation. However, the induction of other nutritional requirements appears to affect the production of the red pigment as well. Nevertheless, since the dissected sample was not a random sample of induced prototrophs but a sample of prototrophs of each color, the color distribution of the colonies is needed for an estimate of the relative proportions of

TABLE 9

A. Meiotic analysis of gamma ray induced recombinants from heteroallelic diploids of the genotype $\frac{+\quad +\quad +2\quad met10}{his2\quad cdc14\quad 1+\quad +}$[1]

	Genotype[2,3]	Event	Color	Number
1A	$\frac{+\ +\ SUP6\ -}{-\ -\ sup6\ +}$	conversion of distal allele	pink	5
2A	$\frac{+\ +\ sup6\ -}{-\ +\ SUP6\ +}$	co-conversion of proximal allele and *cdc14*	pink	3
3A	$\frac{-\ -\ SUP6\ +}{-\ -\ sup6\ +}$	conversion of proximal allele and crossover proximal to *his2*	white	1
4A	$\frac{+\ +\ SUP6\ +}{+\ +\ SUP6\ +}$	complex[4]	white	2
5A	$\frac{+\ -\ SUP6\ -}{+\ -\ sup6\ +}$	complex[4]	pink	1
6A	$\frac{+\ +\ sup6\ +}{-\ -\ SUP6\ -}$	complex, equivalent of meiotic R_2[4]	pink	1
7A	$\frac{+\ +\ sup6\ -}{-\ -\ SUP6\ +}$	conversion of proximal allele	red	1
8A	$\frac{+\ +\ SUP6\ +}{-\ -\ SUP6\ +}$	complex[4]	white	1
9A	$\frac{+\ +\ SUP6\ -}{-\ -\ sup6\ -}$	complex[4]	white	1
10A	$\frac{+\ +\ SUP6\ +}{-\ -\ SUP6\ -}$	complex[4]	white	1
11A	$\frac{+\ +\ SUP6\ +}{-\ +\ sup6\ -}$	complex[4]	pink	1

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[1] *1* and *2* represent secondary mutations of *SUP6-1*.

[2] *SUP6* indicates restoration of the suppressor phenotype.

[3] *sup6* indicates no suppression.

[4] Complex events require two or more independent conversions and/or crossovers.

(Cont'd)

Table 9 (cont'd)

B. Meiotic analysis of gamma ray induced recombinants from heteroallelic diploids of the genotype $\frac{+ \quad + \quad 1+ \quad \mathit{met10}}{\mathit{his2} \quad \mathit{cdc14} \quad +2 \quad +}$[1]

	Genotype[2,3]	Event	Color	Number
1B	$\frac{- \quad - \quad \mathit{SUP6} \quad -}{- \quad - \quad \mathit{SUP6} \quad -}$	complex[4]	white	1
2B	$\frac{+ \quad + \quad \mathit{SUP6} \quad -}{- \quad - \quad \mathit{sup6} \quad +}$	conversion of proximal allele	pink	2
3B	$\frac{+ \quad + \quad \mathit{sup6} \quad -}{- \quad - \quad \mathit{SUP6} \quad +}$	conversion of distal allele	pink	3
4B	$\frac{+ \quad - \quad \mathit{SUP6} \quad -}{- \quad - \quad \mathit{SUP6} \quad +}$	complex[4]	white	1
5B	$\frac{+ \quad - \quad \mathit{SUP6} \quad -}{- \quad - \quad \mathit{sup6} \quad +}$	co-conversion of proximal allele and *cdc14*	pink	1
6B	$\frac{+ \quad + \quad \mathit{sup6} \quad -}{- \quad - \quad \mathit{SUP6} \quad -}$	co-conversion of distal allele and *met10* or crossover between alleles or conversion with associated crossover	white	3
7B	$\frac{+ \quad + \quad \mathit{SUP6} \quad +}{- \quad + \quad \mathit{SUP6} \quad -}$	complex[4]	white	1

TABLE 10

Classification of gamma ray induced prototrophic diploids

	Pink	Red	White
Number of diploids dissected	22	8	12
Mutation to suppressor at a second locus	5	7	-
Conversion of proximal allele	2	1	1
Conversion of distal allele	8	-	-
Co-conversion of proximal allele and *cdc14*	4	-	-
Co-conversion of distal allele and *met10* or crossover between alleles or conversion accompanied by crossover	-	-	3
Complex event. More than one independent conversion and/or crossover	3	-	8

TABLE 11

Color distribution of gamma ray induced prototrophic diploids

	Pink	Red	White
1-7 x *1-7*	.721	.131	.147
1-2 x *1-6*	.810	.107	.083
1-6 x *1-6*	.770	.174	.054
1-7 x *1-2*	.856	.005	.143
1-7 x *1-6*	.848	.001	.151
1-7 x *1-8*	.383	.004	.614
1-7 x *1-5*	.900	.037	.063
1-7 x *AC*	.953	.005	.043
1-7 x *1-3*	.806	.043	.150
1-6 x *1-10*	.859	.023	.117
1-6 x *1-11*	.899	.038	.063
1-6 x *1-5*	.850	.028	.122
1-6 x *AC*	.923	.007	.070
1-6 x *1-3*	.851	.018	.131
1-6 x *1-20*	.927	.007	.066
1-8 x *1-3*	.801	.047	.035
1-10 x *1-3*	.834	.028	.138
1-11 x *1-3*	.867	.019	.115
AC x *1-3*	.882	.014	.104
1-3 x *1-20*	.893	.007	.100

different kinds of events. In 16 of 17 heteroallelic crosses scored, 80% to 95% of the colonies were pink, 4% to 15% white, and 0.1% to 5% red. Only cross *1-7* x *1-8* showed a very different color distribution. The first three crosses of Table 11 were either self-crosses or crosses between alleles not seprable by meiotic recombination, and it may be seen that the proportion of reds increased at the expense of white and pink colonies. The color distribution for each set of diploids was taken from the total of colonies scored at all doses. In the majority of cases, there was no significant change in the relative proportions of pinks, whites and reds with different doses of gamma rays.

The results presented in Tables 10 and 11 indicate that only about 40% of the prototrophs from heteroallelic crosses were simple conversions of one allele of *SUP6*. Sixteen to 18% were most simply explained as co-conversions extending into the next locus, and 20% were complex events in the region of *SUP6*, including conversions with nonadjacent crossovers, two independent conversions, four-strand double crossovers and other less easily classified combinations of events.

Of the 12 strains in which the prototroph was the result of an induced suppressor at a locus other than *SUP6* (Table 10), nine showed loose linkage with *met10* and *cdc14*, and therefore were probably mutations of *SUP11*. They were predominantly red in color as expected from a suppressor considerably less efficient at suppressing *ade2-1* than *SUP6* (Hawthorne and Leupold 1974).

III. Tetrad Analysis

A total of 3345 tetrads were dissected to analyse recombination in the region of *SUP6*. The numbers and types of aberrant tetrads for each allele scored are presented in Table 12.

Conversion to Mutant and to Wild-type

The equality of 3+:1m and 1+:3m segregations for all alleles tested except *met10-4* (Table 12) makes it appear that the frequency of conversion from mutant to wild-type and wild-type to mutant was, with the one exception, equal for all alleles. Because of the small number of postmeiotic segregations, it was impossible in most cases to tell whether postmeiotic segregation of chromatids originally carrying the mutant allele was equal in frequency to postmeiotic segregation of chromatids with the wild-type allele. However, there was an overall equality of 3:5 and 5:3 segregations for the total of all alleles, and for *SUP6-1* the numbers were large enough to make the comparison. From the 3:5, 5:3, aberrant 4:4 and aberrant 2:6 segregations, it was calculated that there were 15 postmeiotic segregations of *SUP6-1*-carrying chromatids and 22 postmeiotic segregations of *sup6*$^+$-carrying chromatids, that is, they were equal.

The significant excess of 3:1 over 1:3 segregations for *met10-4* suggests that there was an inequality of conversion in the two directions. However, this conclusion is put in doubt by evidence from both random spores and incomplete tetrads suggesting selection against methionine auxotrophs.

TABLE 12

Number of tetrads with aberrant segregation for all markers tested

Ratio *wild-type*:mutant	3:1	1:3	4:0	0:4	5:3	3:5	2:6^{ab}	4:4^{ab}	7:1	1:7	Number of tetrads	Percent tetrads with aberrant segregation
his2	182	205	7	8	5	0	0	0	0	0	3115	13.07
cdc14	132	107	3	1	5	2	0	0	0	1	3167	7.93
SUP6-1	128	126	7	7	12	17	1*	3**	2	0	1952	15.52
sup6-1-2	29	31	4	0	3	2	0	0	1	0	333	21.02
sup6-1-3	27	43	0	2	5	5	0	0	1	0	341	24.34
met10-4	92	53	1	2	5	0	0	0	0	0	3063	5.00
ura4-11	23	17	28	22	0	1	0	0	0	0	3409	2.67
leu2	98	96	1	2	1	1	0	0	0	0	3407	5.72

**SUP6-1/sup6*$^+$
SUP6-1/SUP6-1
SUP6-1/sup6$^+$
SUP6-1/SUP6-1

***SUP6-1/SUP6-1*
SUP6-1/sup6$^+$
SUP6-1/sup6$^+$
sup6$^+$*/sup6*$^+$

Among unselected random spores, there was a statistically non-significant excess of methionine prototrophs when the random spore preparation was plated on complete defined medium ($\chi_1^2 = 3.55$; $p > .05$), but a significant excess on YEPD ($\chi_1^2 = 5.47$; $p < .025$), and the sum of these was significant at the 1% level ($\chi_1^2 = 7.67$; $p < .01$). Both *his2* and *cdc14* showed equal numbers of mutant and wild-type spores.

In addition, 900 spores from 375 tetrads in which one or more spores did not germinate showed a significant excess ($\chi_1^2 = 7.84$; $p < .01$) of *MET10* over *met10-4* spores, accompanied by a significant deficiency of *CDC14* and *HIS2* compared to the mutant alleles ($\chi_1^2 = 5.44, 5.13$; $p < .05$). The number of leucine and uracil auxotrophs were equal to the number of prototrophs, and in 678 spores from crosses heterozygous for *SUP6*, the number of suppressor-carrying spores was equal to the number of spores with a mutated suppressor of the wild-type allele at that locus.

A crude estimate was made as to whether the selective death of methionine auxotrophs in incomplete tetrads was sufficient to account for the inequality of conversion to mutant and to wild-type detected in complete tetrads. Average spore viability was about 87%, although there were several sources of inaccuracy in this estimate. For example, while tetrads incompletely dissected for technical reasons were included in this count, asci in which all four spores were inviable were excluded. Furthermore, there was considerable variation between crosses. Nevertheless, using the inequality of *met10-4* and *MET10* spores in incomplete tetrads and the estimate of 13% spore death, it was calculated that 4.8% of *MET10* spores and 21.1% of *met10-4* spores did not germinate in four-spored asci. The probability of all four spores surviving in a + + + - tetrad, where + and - refer to the methionine requirement only, is $(1-.048)^3(1-.211) = .681$. For a - - - + tetrad the probability is

$(1-.048)(1-.211)^3 = .467$. Using this correction, the actual frequency of 3:1 segregations for methionine was the observed frequency divided by the probability of all spores of such a tetrad surviving, that is $.030/.681 = .044$. The actual frequency of 1:3 segregations was $0.17/.467 = .036$. 4.4% and 3.6% of 3063 tetrads are not significantly different from each other ($\chi^2_1 = 2.18$; $p > .05$). Consequently, spore lethality may have been the cause of the inequality.

Lawrence et al (1975) found a similar excess of 3:1 over 1:3 segregations for the *met3* gene in *Saccharomyces*. The *met1-1* allele showed no such inequality in the study of Fogel and Mortimer (1969).

4:0 and 0:4 Ratios

A 4:0, 0:4, 7:1 or 1:7 meiotic segregation implies hybrid DNA formation at overlapping sites on both pairs of non-sister chromatids. However, several spurious sources of these ratios must first be ruled out: false tetrads, reversion, and mitotic recombination.

If the aberrant segregations referred to were the result of picking four-spore aggregates which were not products of a single meiosis (false tetrads), then it is expected that the unlinked genes would have segregated irregularly in most cases. Of 33 tetrads with 4:0 or 0:4 segregation of alleles on linkage group VI, only three showed aberrant segregation of *leu2*, a small sample, but not significantly different from the frequency of *leu2* conversion in the general population of tetrads. *Ura4-11* segregated 2:2 in all 33 cases. This makes it unlikely that many of these "wider ratio" tetrads were the result of false clusters.

There are two factors which rule out mutation as contributing significantly to the wider ratios. The first is the overall equality of 4:0 and 0:4 segregations. Excluding *ura4-11*, which showed an extremely high frequency of all-mutant and all-wild-type tetrads, there were twenty-two 4:0 segregations and twenty-two 0:4's. One would expect that mutation to the mutant phenotype would be more frequent than mutation to wild-type for all markers, except possibly the suppressor, yet the totals for *his2*, *cdc14*, *met10* and *leu2* were eleven 4:0's and thirteen 0:4's.

In addition, the frequency of wider ratios ranged from 9×10^{-4} to 150×10^{-4}. This is much higher than the mutation frequency. A 0:4 or 4:0 segregation in a cross heterozygous for the marker in question would require the occurrence of a mutation before premeiotic DNA synthesis, whether that mutation be at the same locus or at a second site. Self crosses of secondarily mutated suppressors showed a frequency of up to 67 suppressors in 10^6 spores (Table 3) or 17×10^{-6} per tetrad. Furthermore, the majority of these probably arose during meiosis or premeiotic DNA synthesis. The frequency of 4:0 and 0:4 segregations of *SUP6* was 7.6×10^{-3} per tetrad. It is unlikely that differences in the number of diploid cell generations or different growth conditions could account for the nearly three orders of magnitude difference between the frequency of mutation in self crosses and the mutation frequency required to account for the occurrence of all-mutant or all-wild-type tetrads, particularly since the growth of the diploid strains was kept to a minimum for all studies.

Mitotic recombination is perhaps the most difficult spurious cause to rule out. Although growth of diploids was kept to a minimum, it is not known how many diploid cell generations there were before sporulation, and this figure is needed in order to estimate the frequency of mitotic recombination. Because a variety of events accompany or are a part of spontaneous mitotic conversion (Kakar 1963; Hurst and Fogel 1964), one cannot use the lack of distal homozygosis as evidence for the lack of mitotic recombination. However, mitotic gene conversion and crossover are not expected to generate 7:1 and 1:7 segregations, and if these were of the same origin as the 4:0 and 0:4's, their origin was probably meiotic.

The expected wider ratio frequencies were calculated from observed tetrad frequencies using the partly corrected formulae of Lamb and Wickramaratne (1973). These are based on the assumption that the frequency of hybrid DNA formation on two pairs of non-sister chromatids at a point is determined by the probability of hybrid formation on one pair of chromatids (taken from the 6:2, 2:6, 5:3 and 3:5 ratios). The partially corrected formulae correct the calculations for the occurence of hybrid DNA at corresponding sites on both pairs of non-sister chromatids when these lead to an 8:0, 0:8, 7:1 or 1:7 segregation.

$$\text{Expected}\quad 8{:}0 = (\tfrac{1}{2}6{:}2 + 8{:}0 + \tfrac{1}{2}7{:}1)^2$$

$$0{:}8 = (\tfrac{1}{2}2{:}6 + 0{:}8 + \tfrac{1}{2}1{:}7)^2$$

$$7{:}1 = 2(\tfrac{1}{2}6{:}2 + 8{:}0 + \tfrac{1}{2}7{:}1)(\tfrac{1}{2}5{:}3 + \tfrac{1}{2}7{:}1)$$

$$1{:}7 = 2(\tfrac{1}{2}2{:}6 + 0{:}8 + \tfrac{1}{2}1{:}7)(\tfrac{1}{2}3{:}5 + \tfrac{1}{2}1{:}7)$$

Table 13 shows an approximately twofold excess of observed to expected tetrads in which recombinants were formed on the two pairs of non-sister chromatids at corresponding sites.

ura11 was not included in the totals because of the extremely high frequency of 4:0 and 0:4 segregations. It is not known what caused this high frequency. This gene is the distal-most marker on linkage group XII, as determined by mitotic linkage, but is meiotically unlinked to any other marker. A high frequency of 0:4 and 4:0 segregations of *ura4-11* has been observed before in a different genetic background (von Borstel, unpublished).

Variation in Conversion Frequencies

From Table 12 it can be seen that alleles *sup6-1-2* and *sup6-1-3* showed a higher frequency of tetrads with non-Mendelian segregation than *SUP6-1*; the smaller difference was significant at the 0.5% level. Table 14 shows that this was not peculiar to the *SUP6* locus, but that conversion of *cdc14* and *met10* also increased in crosses A75 and A76. For *met10* there was an increase both in the frequency of *SUP6* and *met10* co-conversion and in the frequency of *met10* single-site conversion in these crosses. For *cdc14* the increase was only in co-conversions. All other classes of cross were homogeneous with respect to the amount of conversion of *his2*, *cdc14*, and *met10*.

TABLE 13

Observed and expected number of "wider ratio" tetrads

		8:0	0:8	7:1	1:7
his2	Observed	7	8	0	0
	Expected	3.05	3.74	.16	0
cdc14	Observed	3	1	0	1
	Expected	1.50	.95	.11	.05
SUP6-1	Observed	7	7	2	0
	Expected	2.65	2.62	.59	.61
sup6-1-2	Observed	4	0	1	0
	Expected	1.08	.72	.23	.09
sup6-1-3	Observed	0	2	1	0
	Expected	.57	1.62	.25	.35
met10	Observed	1	2	0	0
	Expected	.74	.74	.02	.02
leu2	Observed	1	2	0	0
	Expected	.74	.74	.02	.02
Total	Observed	23	22	4	1
	Expected	10.31	10.56	1.43	1.12
ura4-11	Observed	28	22	0	0
	Expected	.45	.28	0	.01

TABLE 14

Effect of *SUP6* background on conversion of linked loci

Background	Diploid numbers	Percent aberrant segregations *his2*	*cdc14*	*met10*	Number of tetrads
1. $\frac{\mathit{his2}\quad \mathit{cdc14}\quad \mathit{sup6}^{+}\quad +}{+\quad +\quad \mathit{SUP6\text{-}1}\quad \mathit{met10}}$	A50, A51, A52, A53, A011, A021, A022, A031, A032, A041, A042, A811, A161, A181, A182, A183	12.91	7.38	4.25	1952
2. $\frac{\mathit{his2}\quad \mathit{cdc14}\quad \mathit{sup6\text{-}1\text{-}x}\quad +}{+\quad +\quad \mathit{SUP6\text{-}1}\quad \mathit{met10}}$	A75 A76	14.39	11.13	8.38	674
3. $\frac{\mathit{his2}\quad \mathit{cdc14}\quad \mathit{sup6}^{+}\quad +}{+\quad +\quad \mathit{sup6}^{+}\quad \mathit{met10}}$	A15	9.40	4.70	1.34	149
4. $\frac{\mathit{his2}\quad \mathit{cdc14}\quad \mathit{SUP6\text{-}1}\quad +}{+\quad +\quad \mathit{SUP6\text{-}1}\quad \mathit{met10}}$	A100	15.50	6.95	5.35	187
5. $\frac{\mathit{his2}^{*}}{+}$	A16, A17	9.80	-	-	153
6. $\frac{\mathit{cdc14}^{*}}{+}$	A18, A21	-	6.34	-	205
7. $\frac{\mathit{met10}^{*}}{+}$	A19	-	-	1.98	101

*All other markers on linkage group VI homozygous *wild-type*.

Variation in Postmeiotic Segregation Frequencies of Different Alleles of *SUP6*

Of 1952 tetrads dissected from diploids of the genotype *SUP6-1/sup6*$^+$, 35 or 1.8% showed postmeiotic segregation of *SUP6*, although there were actually 39 spores with such an event. There were 1.8% or 6 postmeiotic segregations in 333 tetrads from the cross involving *sup6-1-2*, and 11 in 341 for *sup6-1-3* (3.2%). None of these frequencies is significantly different from the others. Among those tetrads which showed either postmeiotic segregation or conversion, 9% to 14% of those events were postmeiotic segregations and again comparison of the three kinds of cross showed no significant differences. In this sample of three *SUP6* alleles, one of which (*sup6-1-2*) behaved as a deletion, the frequency of postmeiotic segregation per spore or relative to the conversion frequency did not vary significantly.

Outside Marker Recombination

The analysis of 2626 complete tetrads from crosses heterozygous for *SUP6* showed that the parental combination of outside markers exceeded the recombinant among *SUP6* convertants. This is analogous to the situation observed in the random meiotic products from two-point crosses in which the intragenic *SUP6* recombinants were more often of the parental class than the recombinant. The crosses in which tetrad analysis was performed were of three types:

1) *his2 cdc14* + + x + *SUP6-1 met10*
2) *his2 cdc14* + + x + *SUP6-1 met10* in a homozygous radiation sensitive background
3) *his2 cdc14 sup6-1-x* x + + *SUP6-1 met10*.

Tables 15, 16, and 17 show the number of conversions of *SUP6* alone, co-conversions of *SUP6* and *cdc14* and conversions of *cdc14* alone, classified as to whether these were or were not accompanied by outside marker exchange involving the converted chromatid. Conversion of *SUP6* alone showed 37% associated recombination of outside markers. The difference from 50% was highly significant ($\chi_1^2 = 20.2$; $p << .001$). For that same set of tetrads, *cdc14* showed 50% flanking marker recombination among convertants, and the longer co-conversion events involving both loci showed an excess of recombinant outside markers ($\chi_1^2 = 8.6$; $p < .005$). Total conversions of *SUP6*, including conversions of *SUP6* alone and co-conversions with *cdc14*, still showed a significant excess of non-crossover tetrads, 205 *SUP6* convertants with parental flanking markers and 154 with the recombinant class, that is 43% outside marker exchange ($\chi_1^2 = 7.2$; $p < .01$).

One can estimate the contribution of unrelated crossovers which affected the converted chromatid since these should equal the number of crossovers in regions adjacent to the converted length which were detected because they did not involve the converted chromatid. It will be seen from Tables 15 and 17 that there were only six such events in the parental class and seven in the recombinant class. Consequently, any correction for unrelated crossovers is small and goes equally in the two directions.

Only those events which did not involve co-conversion of a flanking

TABLE 15

Configuration of outside markers in spores with conversion or postmeiotic segregation of *SUP6*

Genotype	Diploid	Number of tetrads	PARENTAL: Number of conversions with parental outside markers	PARENTAL: Unrelated crossover between *cdc14* and *met10*	PARENTAL: Unrelated crossover between *his2* and *cdc14*	RECOMBINANT: Number of conversions with recombinant outside markers	RECOMBINANT: Unrelated crossover between *cdc14* and *met10*	RECOMBINANT: Unrelated crossover between *his2* and *cdc14*	NOT DETERMINED: Conversion of flanking marker	OMITTED: 0:4 or 4:0 segregation for *SUP6*
SUP6-1 x *sup6*$^+$										
1. *RAD*$^+$	A50	149	6			4			3	
RAD$^+$	A51	148	11		1	4			2	
RAD$^+$	A52	130	10			2			4	
PAD$^+$	A53	19	2			1			0	
2. *rad1-15*	A811	178	9		1	8	1	1	0	1
rad1-5	A011	166	10			4			0	
rad2-6	A021	172	8			2	1	1	1	
	A022	110	10		1	3			1	
rad3-2	A031	104	7	1	1	4	1	1	2	
	A032	145	8		1	8	1		1	1
rad4-4	A041	140	10	1	1	6			1	1
	A042	52	4			2			1	
rad16-1	A161	157	10		2	2			1	1
rad18-1	A181	60	5	1	1	3			2	
	A182	90	9		1	10		1	0	
	A183	132	10	1		6		1	1	1
sup6-1-x x *SUP6-1*										
3. *sup6-1-3*	A75	341	22	1		25		1	9	2
sup6-1-2	A76	333	30	1	3	11	–	1	3	3
			181	6	13	105	4	7	32	10

TABLE 16

Configuration of outside markers in spores with co-conversion of *SUP6* and *cdc14*

			PARENTAL		RECOMBINANT		NOT DETERMINED	OMITTED
Genotype	Diploid	Number of tetrads	Number of conversions with parental outside markers	Unrelated crossover between *his2* and *met10*	Number of conversions with recombinant outside markers	Unrelated crossover between *his2* and *met10*	Conversion of flanking marker	0:4 or 4:0 segregation for *SUP6* and/or *cdc14*
SUP6-1 x *sup6*$^+$								
1. *RAD*$^+$	A50	149	3		5		1	
RAD$^+$	A51	148	0		2		3	
RAD$^+$	A52	130	1		2		2	
RAD$^+$	A53	19	0		0		0	
2. *rad1-18*	A811	178	2		3		2	1
rad1-5	A011	166	1		0		1	
rad2-6	A021	172	1		0		2	2
	A022	110	1		2		1	1
rad3-2	A031	104	0		1		2	
	A032	145	1		1		2	
rad4-4	A041	140	0		6		3	1
	A042	52	2		1		3	
rad16-1	A161	157	0		0		2	
rad18-1	A181	60	0		1		0	
	A182	90	1		1		1	1
	A183	132	3		5		1	2
sup6-1-x x *SUP6-1*								
3. *Sup6-1-3*	A75	341	6		10		9	1
Sup6-1-2	A76	333	2		9	1	12	1
			24	0	49	1	47	10

TABLE 17

Configuration of outside markers in spores with conversion of *cdc14*

Genotype	Diploid	Number of tetrads	PARENTAL: Number of conversions with parental outside markers	PARENTAL: Unrelated crossover between *his2* and *SUP6*	RECOMBINANT: Number of conversions with recombinant outside markers	RECOMBINANT: Unrelated crossover between *his2* and *SUP6*	NOT DETERMINED: Conversion of flanking marker	OMITTED: 0:4 or 4:0 segregation for *cdc14*
SUP6-1 x *sup6*$^+$								
1. *RAD*$^+$	A50	149	2		1		1	
RAD$^+$	A51	148	0		3		0	
RAD$^+$	A52	130	5		1		2	
RAD$^+$	A53	19	0		0		1	
2. *rad1-18*	A811	178	1		3		1	
rad1-5	A011	166	2	1	1		0	
rad2-6	A021	172	1	1	0		1	
	A022	110	1		0		0	
rad3-2	A031	104	2		3		1	
	A032	145	1		2		1	
rad4-4	A041	140	0		2		2	
	A042	52	2		1		0	
rad16-1	A161	157	2		1		0	
rad18-1	A181	60	1		2		1	
	A182	90	0		3		0	
	A183	132	3		3	1	1	
sup6-1-x x *SUP6-1*								
3. *sup6-1-3*	A75	341	12		3		1	
sup6-1-2	A76	333	0		2		6	
			35	2	31	1	19	0

marker were tabulated here, since co-conversion and often independent conversion of these makes it impossible to tell whether or not the event was associated with a crossover. The 4:0 and 0:4 segregations were also omitted from this analysis, but would have had little effect on the results if included. Of nine such tetrads in which the markers flanking *SUP6* could be determined, six showed a single crossover in the *cdc14* to *met10* region, that is six conversions in 18 from these tetrads were accompanied by a crossover.

Effect of Spore Death on the Frequency of Outside Marker Recombination

Since conversion can be detected only in complete tetrads, the possibility exists that asci with inviable spores included a higher proportion of convertants with associated crossovers than the complete tetrads did. In the 900 spores from 375 incomplete tetrads there was a significant deficiency of one of the parental combinations of *cdc14* and *met10*, but no significant difference between the two recombinant combinations. So crossover tetrads did not appear to have been lost by a relative inviability of one recombinant genotype. The invomplete tetrads were re-examined to determine the proportion which contained a spore recombinant in the *cdc14-met10* region. A higher proportion of the incomplete tetrads had a recombinant product than did the complete tetrads. If conversion with crossing-over sometimes caused cell death, recombinant *SUP6* convertants might have been lost from complete tetrads. It is therefore possible that the inequality of parental and recombinant classes among *SUP6* convertants was due to the event itself (conversion of *SUP6* with crossing-over) generating an inviable spore. However, of 39 three-spored incomplete tetrads with recombination in the *cdc-met* interval, ten of these contained reciprocal recombination products. Had conversion of *SUP6* with crossing-over often resulted in the production of an inviable product, fewer incomplete tetrads with reciprocal products should have been recovered. While this reasoning argues against the event having been lethal, the large number of unknowns in the data make it impossible to exclude this possibility.

Length of Conversion Events

Table 18 shows the number of postmeiotic segregation or conversion events involving *SUP6* alone or covering adjacent loci as well. Included as co-convertants are co-postmeiotic segregants and conversions in one locus accompanied by postmeiotic segregation in the adjacent locus on the same chromatid. The totals for all the crosses show that 32.3% of all events at *SUP6* extended outside the boundaries of that gene in one or both directions, and that co-conversion sometimes spanned the length of the marked region, that is, a minimum of four loci. The frequency of these long events was far too high to have been the result of coincident but independent conversion of the flanking markers. 35% of all conversions of *met10*, 59% of all conversions of *cdc14*, and 8% of conversions of *his2* involved co-conversion of *SUP6*. This was not an allele specific effect of the original suppressor mutation, but was characteristic of conversion involving secondary mutations at this locus as well. Both the high conversion frequency and the characteristically long conversion

TABLE 18

Conversion of *SUP6* and co-conversion of adjacent loci

		Frequency of tetrads with conversion or postmeiotic segregation at *SUP6*	Number of events covering *SUP6* alone	*SUP6* + *cdc14*	*SUP6* + *met10*	*SUP6* + *cdc14* + *met10*	*SUP6* + *cdc14* + *his2*	*SUP6* + *cdc14* + *his2* + *met10*
SUP6-1 x *sup6*$^+$								
	A50	.148	11	8	2	1	-	-
	A51	.148	16	3	1	-	2	-
	A52	.162	15	3	1	1	1	-
rad1-18	A811	.142	18	5	-	1	1	-
rad1-5	A011	.096	14	2	-	-	-	-
rad2-6	A021	.076	9	1	1	-	2	-
	A022	.156	12	3	1	1	-	-
rad3-2	A031	.154	12	1	1	2	-	-
	A032	.146	16	2	1	-	2	-
rad4-4	A041	.188	17	6	-	1	1	1
	A042	.269	8	3	-	1	2	-
rad16-1	A161	.096	13	-	-	2	-	-
rad18-1	A181	.183	9	1	1	-	-	-
	A182	.236	18	2	-	-	1	-
	A183	.202	16	8	1	-	-	1
sup6-1-3 x *SUP6-1*	A75	.239	49	18	7	3	2	2
sup6-1-2 x *SUP6-1*	A76	.201	40	12	3	4	3	4
			293	78	20	17	17	8

lengths are locus- or region- but not allele-specific phenomena. An indication of the amount of co-conversion is given in Figure 7, which shows the percent tetrads with single site and co-conversions for this region. The 4:0 and 0:4 segregations were omitted from this analysis.

Interference Between Conversion Events

Interference between conversions was checked for all pairs of markers. The expected and observed numbers of coincident conversions are found in Table 19. For unlinked genes, the expected frequency is simply the product of the observed conversion or postmeiotic segregation frequencies at each locus. For linked loci, the occurrence of co-conversion must be taken into account. When looking at interference, one is interested in coincident but independent events. An event is scored as a co-conversion if there has been conversion in the same direction on the same chromatid of contiguous markers. For the markers on linkage group VI, the co-conversion frequency for a given pair of loci was subtracted from the total conversion frequency for each locus before the two were multiplied to calculate the expected frequency of coincident events. The observed number of coincident events for two adjacent markers came from tetrads in which one locus was converted to mutant and the other to wild-type, or both were converted in the same direction but on different chromatids. If the two linked loci were not adjacent, the absence of an event covering the intervening marker automatically classified two conversions as coincident conversions and not as a co-conversion, whether or not they were in the same direction or on the same strand. The 4:0 and 0:4 segregations were omitted from this analysis.

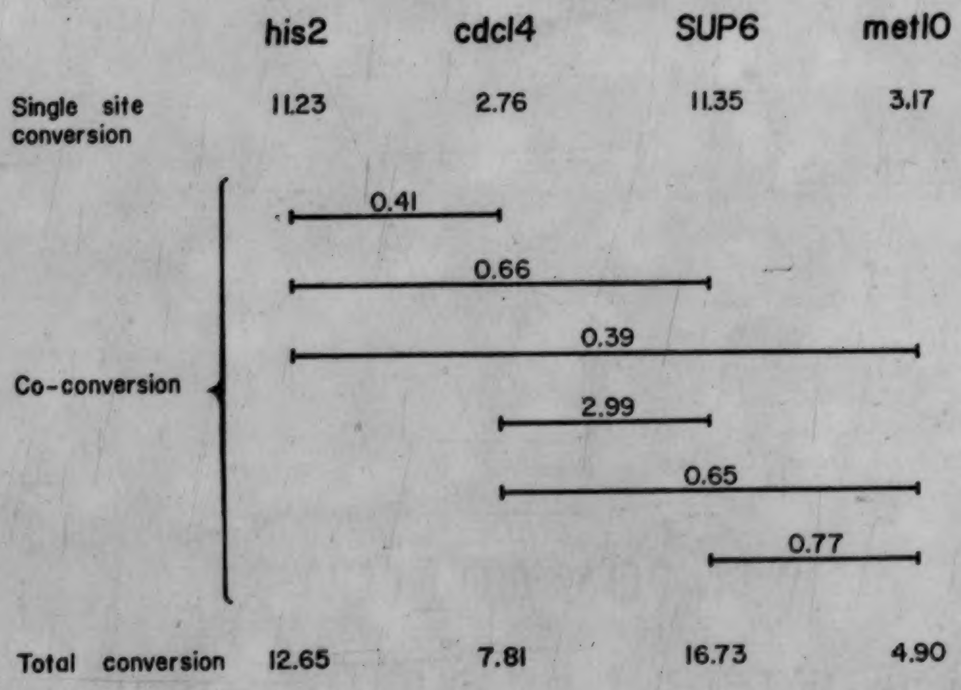
his2
cdcl4
SUP6
met10
Single site conversion
11.23
2.76
11.35
3.17
0.41
0.66
0.39
Co-conversion
2.99
0.65
0.77
Total conversion
12.65
7.81
16.73
4.90

Fig. 7. Percent tetrads with single and multiple site conversions.

TABLE 19

Observed and expected numbers of coincident conversions

	Observed number	Expected number
leu2 - *his2*	19	22.44
leu2 - *cdc14*	17	14.16
leu2 - *SUP6*	32	24.93
leu2 - *met10*	14	8.58
leu2 - *ura4*	7	2.34
ura4 - *his2*	7	4.68
ura4 - *cdc14*	4	2.95
ura4 - *SUP6*	7	5.19
ura4 - *met10*	6	1.80
	113	87.07
	χ^2 = 3.4; p = .05 - .10	
his2 - *cdc14*	15	21.62
his2 - *SUP6*	39	47.78
his2 - *met10*	15	16.70
cdc14 - *SUP6*	10	10.10
cdc14 - *met10*	15	8.13
SUP6 - *met10*	17	12.40
	111	116.73
	χ^2 = .38; p = .50 - .70	

On the assumption that conversions show neither positive nor negative interference, no pair of markers showed a significant difference between observed coincident events and expected events. Although there appeared to be a slight but consistent excess of coincident events between unlinked markers, the sum of all observed double events did not show a significant difference from the expected. For the linked markers, a correction should be made for the 1/8 of independent conversions which would have been falsely scored as co-conversions. Decreasing the expected number by 1/8 undetectable events did not alter its near equality with the observed. Further correction which subtracts the independent events which appeared as co-conversions from the observed number of co-convertants in order to re-estimate the expected frequency of independent events is a refinement beyond the powers of resolution of the data. Because the expected number of independent events which appeared as co-conversions was very small, the results of the previous section are not significantly affected.

False Tetrads

The lack of a significant positive association of independent conversion events as well as the following study of segregation of the mating-type alleles argue against false tetrads having contributed significantly to this study of *SUP6*. As was discussed earlier, the dissection of four-spore aggregates not products of a single meiosis is expected to give frequent aberrant segregation at more than one locus. In the cross *sup6-1-3* x *SUP6-1*, tetrads with conversion of one of the regularly scored markers were tested for segregation of mating type. Scoring was done microscopically by looking for zygotes in a mixture of the colony being tested and a tester strain of each mating type. Of 100 tetrads in which all four spores were scored, all showed 2:2 segregation for mating type. Six tetrads were incompletely scored because of poor mating of one or more spores. On the least favorable assumption that false tetrads result from the dissection of three spores from one tetrad and one unrelated spore, 50% of false tetrads are expected to show aberrant segregation for any one marker. Since this study showed no aberrant segregation for mating type in 100 tetrads, it may be stated with 95% confidence that there were fewer than 6% false tetrads among asci which showed conversion for *his2*, *cdc14*, *SUP6*, *met10*, *ura4* or *leu2*. The genotypes of the spores not scored for mating type made it highly unlikely that they were diploid; they all showed 2:2 segregation for at least three other markers.

Associated Conversion

The lack of positive correlation between conversions in the *his2* to *met10* interval on linkage group VI would suggest that there was not a significant amount of independent correction of two markers included in the same segment of hybrid DNA. However, if the extent of hybrid DNA were unequal on the two chromatids, independent correction of two adjacent loci on one chromatid would not be detected. It would be seen either as co-conversion or as conversion of one locus and a return to the parental genotype at the other, that is a single-site conversion. In this system, independent correction of hybrid DNA on one chromatid might be detected if conversion of non-adjacent sites involved the same chromatid more often than expected by random association. Of 34 *his2 SUP6* conversions in the same tetrad, only five were on the same strand compared to 8.5 expected if the two were independent. In six *cdc14 met10* coincident conversions and seven *his2 met10* coincident conversions, all involved more than one chromatid. At least over the span of three to four genes, there was no preference for conversions on the same strand. The observed negative association between events on one strand was not significant.

Postmeiotic Segregation and Associated Conversion

Of 120 events scored as co-conversions of *cdc14* and *SUP6*, ten involved conversion of *cdc14* and postmeiotic segregation of *SUP6*. For *SUP6* and *met10*, four tetrads in 45 showed conversion of *met10* and postmeiotic segregation of *SUP6*. It is estimated that only one or two of these were independent events. From this we may conclude that mismatch correction did not extend over the entire hybrid length in at least 8%

of the events covering *cdc14* and *SUP6* or *met10* and *SUP6*.

One versus Two Chromatid Events

On the basis of models of hybrid DNA formation and mismatch correction, tetrads which show 3:5 or 5:3 segregation can be used to determine the extent to which heteroduplex DNA is symmetrical, that is, the extent to which it covers both chromatids at the site in question. The classes of asci which must be considered show postmeiotic segregation of one spore with outside markers in the parental configuration. The tritype ascus has three spores with all markers still parental plus one spore containing a postmeiotic segregant (for example the tetrad: A + B, A $\frac{+}{m}$ B, a mb, a m b). This may arise from hybrid DNA on one chromatid only or hybrid on two chromatids with a correction event which returns the hybrid on the second chromatid back to the parental genotype. The tetratype ascus has one spore of each parental genotype, one showing postmeiotic segregation, and one with conversion of the same marker (for example the tetrad: A + B, A $\frac{+}{m}$ B, a + b, a m b). A tetratype ascus requires hybrid DNA to have covered the middle site on both chromatids. Twice the frequency of tetratype asci gives an estimate of the amount of hybrid on both chromatids. In 44 tetrads with 3:5 or 5:3 segregations for *SUP6*, only 13 were free of events covering adjacent loci and parental for flanking markers. All 13 were tritype asci. Three more tetrads with conversion of *cdc14* accompanying the postmeiotic segregation at *SUP6* were also tritype. This suggests a high degree of asymmetry of hybrid DNA in the region of *SUP6*. About 15 postmeiotic segregation events involved co-conversion or co-postmeiotic segregation of flanking markers, and of these approximately one-third would have required the involvement of two chromatids, not necessarily at *SUP6* but within the *his2-met10* region. However, the occurrence of events involving two pairs of non-sister chromatids and the number of alternative ways in which the more complex events could have arisen makes it impossible to quantitate the amount of the asymmetry. Even the three aberrant 4:4 tetrads which are expected to have arisen from the absence of correction on both hybrid strands of a symmetrical event were complex events involving extensive co-conversion and one of these aberrant 4:4's required the participation of all four strands.

Crossover Interference

The frequency of single, double and triple exchanges in a region will reveal whether the occurrence of one crossover interferes with the coincident occurrence of another. However, in this system, nearly 18% of tetrads showed conversion of the outermost markers *his2* and/or *met10*. It is expected that these conversions were often accompanied by crossovers which are not detectable, and therefore a significant proportion of crossover events could not be scored. However, two pieces of evidence suggest a lack of or very low crossover interference in this region. The first is seen in Tables 15, 16, and 17. Among conversions of *SUP6* and/or *cdc14* with parental outside markers, eight or 3.3% were accompanied by an unrelated crossover between the flanking markers which did not involve the converted chromatid. Among convertants with recombinant outside markers, six or 3.2% had a second independent crossover in that same region. A second piece of evidence concerns the occurrence of independent crossovers not involving conversion. The expected number of tetrads showing none, one, two or three independent crossovers was calculated from the mean number of independent crossovers, using the Poisson distribution to determine the probability of each class. The frequency of no events, single, double and triple crossovers fits a Poisson distribution very closely ($\chi^2_2=1.38$; p=.5), indicating no crossover interference.

Map Distances

Figure 8 shows the map distances between loci on linkage group VI given in per cent recombination in complete unselected tetrads analysed as single spores. The numbers in parentheses are map distances calculated from unselected random spores. In all intervals, the amount of recombination was significantly higher in the random spore population. The most obvious possibility is that this was the result of selection against certain genotypes in the random spore population. For example, there was a noticeable deficiency of *his2 cdc14 met10* spores compared to the *HIS2 CDC14 MET10* class in the products of the random spore analysis. These two genotypes are recombinant for *cdc14* and *met10*, but parental for *his2* and *cdc14*. Therefore selection against the triple mutant could not explain the increase in the recombination in the *cdc14 met10* interval. Conversely, the presence of diploids among the random spores giving an excess of + + + colonies is insufficient explanation for the expanded *his2-cdc14* region. Furthermore, haemocytometer counts of the spore suspension showed little or no contamination from vegetative cells. However, a combination of small differences in viability of the 16 possible genotypes could have been responsible for differences in the proportions of recombinant products in tetrads and random spores.

Random spore analysis, of course, includes four-spored asci with one or more inviable spores which were not in the tetrad analysis. However, the recombination frequencies in those incompletely viable

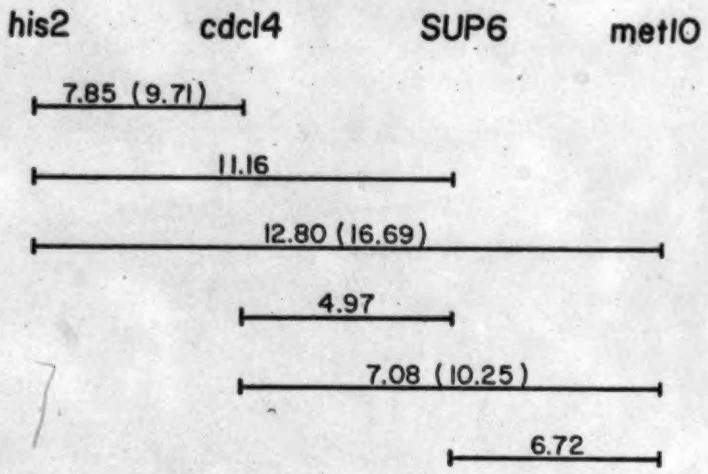
his2
cdcl4
SUP6
met10
7.85 (9.71)
11.16
12.80 (16.69)
4.97
7.08 (10.25)
6.72

Fig. 8. Map distances in percent recombination. Values are from complete unselected tetrads. Numbers in parentheses are map distances from unselected random spores.

tetrads analysed suggest that their inclusion could not have brought the tetrad-derived map distances up to the random spore distances.

Asci formed with one, two, and three spores will also have contributed to the random spore data. Genetic studies of two- and three-spored asci in *Saccharomyces cerevisiae* have consistently shown the spores to be haploid products of meiosis (Bevan 1953; Takahashi 1962; Takahashi and Akamatsu 1963; Esposito et al. 1974). In the study of Esposito et al., performed in a *spo3* background, the two spores were a random sample of the four meiotic products, but the results of Elizabeth Savage (unpublished results from this laboratory) indicate that the spores of a two-spored ascus were preferentially non-sister spores. Her study was done in a wild-type background. In either case, intergenic recombination frequencies should not be affected by the inclusion of products from two-spored asci. However, thers findings indicate that not all systems are identical, and in this study of *SUP6*, no testing of incomplete asci was done.

One further possibility is that genetic differences in the crosses contributed to the discrepancy in the two maps. The random spore data came from crosses in which both parents carried either a secondary mutation of *SUP6-1* or the *sup6*$^+$ allele. This makes the nature of the heterozygosity at *SUP6* slightly different from those crosses in which tetrads were dissected. Since the construction of these strains followed slightly different routes, the general genetic background differed as well.

For both sets of data, the map distances are not additive. Because so much recombination in this region results from conversion and conversion-associated crossing-over, non-additivity of intergenic maps is expected. Conversion of gene B in the sequence A-B-C, accompanied by crossing-over 50% of the time, will yield an equal number of A-B, B-C and A-C recombinant products. This component of recombination is not distance-dependent and will tend to contract long intervals compared to the sum of the sub-segments of that interval.

Effect of Repair Defects on Postmeiotic Segregation

If the enzymatic steps involved in the excision of pyrimidine dimers from UV-irradiated DNA overlapped with those responsible for the recognition and excision of mismatched bases, one would expect strains with defects in dimer excision-repair capacity to show an increase in postmeiotic segregation at the expense of conversion.

The radiation-sensitive mutants *rad1*, *rad2*, *rad3*, and *rad4* were chosen for analysis on the basis of evidence that they control steps in an excision-repair process. After UV irradiation, mutants at these four loci show little loss of pyrimidine dimers from their DNA under conditions in which wild-type strains excise dimers (Unrau et al. 1971; Resnick and Setlow 1972; Wheatcroft 1973). The behavior of double mutants also places these mutations in the same repair pathway, since the survival characteristics of the double mutants are like those of one or the other of the single-mutant parents (Cox and Game 1974). The mutant *rad16* has not been studied biochemically, but it retains photoreactivability after post-irradiation incubation, that is, dimers are not

TABLE 20

Analysis of conversion and postmeiotic segregation in radiation-sensitive strains

Genotype Diploid strain number	Number of tetrads	Number of tetrads with conversion at *SUP6*	Number of tetrads with postmeiotic segregation at *SUP6*	Per cent aberrant tetrads with post-meiotic segregation	Conversion: postmeiotic segregation rad^- genotype compared to control	Aberrant: Mendalian segregation rad^- genotype compared to control
Wild-type						
A50	149	17	6			
A51	148	21	1			
A52	130	18	3			
	427	56 (13.1%)	10 (2.3%)	15.2%		
rad1-18						
A811	178	25 (14.0%)	2 (1.1%)	7.4%	1.02	.01
rad2-6						
A021	172	11	4			
A022	110	16	2			
	282	27 (9.6%)	6 (2.1%)	18.2%	.149	1.99
rad3-2						
A031	104	15	1			
A032	145	20	2			
	249	35 (14.5%)	3 (1.2%)	8.6%	1.16	.01
rad4-4						
A041	140	28	0			
A042	52	11	3			
	192	39 (20.3%)	3 (1.6%)	7.1%	1.55	3.79
rad16-1						
A161	157	15 (9.6%)	1 (.6%)	6.3%	.88	2.64
rad18-1						
A181	150	26	7			
A183	132	26	3			
	282	52 (18.4%)	10 (3.5%)	16.1%	.02	4.89

lost from the DNA during incubation. *rad18* and *rad 51* were chosen as loci which control first steps in alternate repair pathways (Cox and Game 1974); however, the strain homozygous for *rad51-1* sporulated very poorly or not at all depending on the particular diploid used. The spores which were formed were generally inviable.

Table 20 shows that in none of the radiation-sensitive strains tested were there a significant number of conversions lost to postmeiotic segregation. Chi-square values were derived from analysis of two by two contingency tables of conversion and postmeiotic segregation in wild-type and radiation-sensitive strains.

These data can also be used to exclude the possibility that any of the *rad*$^-$ genes tested are involved in a recombination pathway in which failure to carry out a particular step would result in a return to the parental genotype. There was no significant loss of total aberrant segregations or of conversions in the radiation-sensitive strains. The frequency of tetrads with aberrant segregation at *SUP6* was somewhat elevated in strains carrying *rad4-4* and *rad18-1*. The frequency of conversion of *his2*, *cdc14* or *met10* in *rad4-4* and *rad18-1* strains was not significantly different from that in the control strains.

If the frequency of tetrads with conversion, postmeiotic segregation and normal segregation at *SUP6* is compared for all crosses, the total heterogeneity is significant. However, heterogeneity among crosses with the same radiation-sensitive background was less (χ^2_{12} = 19.04; p = 0.1-.05) than the heterogeneity among different radiation-sensitive backgrounds (χ^2_{12} = 25.83; p = .025-.01).

DISCUSSION

Recombination in Radiation-sensitive Strains

The results of the study on radiation-sensitive strains of *Saccharomyces* indicate that while the excision of pyrimidine dimers from UV-irradiated DNA may provide an analogy for the excision of mismatched bases in recombination, mismatch repair does not share those functions coded for by *RAD1, RAD2, RAD3, RAD4,*, *RAD16* or *RAD18* genes. Strains carrying *rad1, rad2, rad3* and *rad4* mutations were examined by Snow (1968) for the frequency of intragenic and intergenic recombination. He found no significant differences in the frequencies of recombination between these strains and wild-type (radiation resistant) controls, although there were some significant frequency differences among the radiation-sensitive strains. However, his system would not have been able to detect changes in correction efficiency, which was specifically the object of this study, nor changes in conversion lengths, outside marker recombination, and other parameters of recombination detectable in the *SUP6* system.

In this study, amount of heterogeneity in conversion, postmeiotic segregation and Mendelian segregation at *SUP6* among strains with different radiation-sensitive backgrounds suggests that either recombination patterns are slightly modified by certain repair defects, or that the genetic background introduced with these defects caused some changes in conversion frequencies at *SUP6*. However, the magnitude of the changes suggests that the products of these *rad* genes play a minor role, if any at all, in recombination. It is possible that under certain conditions, for example, if an enzyme primarily concerned with recombination were defective, these repair enzymes could become important.

However, in the normal functioning of the cell, the steps which were blocked in these strains do not appear to be steps which overlap with the process of recombination. There are other repair enzymes, for example that coded for by *RAD51*, which may be involved in meiotic recombination. However, if their role in recombination is indispensable, their loss results in meiotic lethality.

Characteristics of Induced Mitotic Recombination

Most intragenic mapping in *Saccharomyces cerevisiae* has made use of the method of Manney and Mortimer (1964) who found the induction of prototrophs in a heteroallelic diploid to be linearly related to the dose of X-rays. They used the number of prototrophs per survivor per rad as a measure of distance between alleles. The method has been extended to include the use of gamma rays, sunlamp radiation (Lawrence and Christiansen 1974) and methylmethanesulfonate (Snow and Korch 1970) as mitotic recombination-inducing agents. A comparison of maps generated using different recombinogens is found in Korch and Snow (1973) and Lawrence and Christiansen (1974). In some cases the technique of induction of mitotic recombination has resulted in the construction of clear-cut, highly additive maps (Manney and Mortimer 1964; Tauro et al.1974), but in other studies there were large deviations from additivity (Parker and Sherman 1969; Thuriaux et al. 1971). Fink and Styles (1974) found X-ray mapping of the *his4* region to be reliable only when frequencies were greater than 1.5 prototrophs/10^6/200r. Moore and Sherman (1975) have devoted an extensive discussion to the pitfalls of equating X-ray mapping units with physical distance and to the problems involved in ordering alleles by the rates of X-ray induced mitotic recombination, unless a large number of allelic combinations are tested. Despite the relatively large number of crosses included in this study, *SUP6* was immappable by this technique. The rates of prototroph induction for *SUP6* ranged from 0.026 to 0.532 prototrophs/10^6 survivors/200r (Table 8), which, on the average, was lower than any published map.

The dissection of the gamma ray-induced recombinants suggests that up to one-third of the conversions of *SUP6* may have been co-converted at an adjacent locus (Tables 10 and 11). Long hybrid and excision-repair lengths at this locus seem to be characteristic of induced mitotic as well as meiotic recombination. Another 20% of the prototrophs arose from multiple events for which we have no simple model, and consequently no basis for deciding whether these events were distance-dependent. Many of these required the participation of three or more chromatids. It appears that induced mitotic recombination events in this region were generally more complex than meiotic events.

Other studies in *Saccharomyces* by Hurst and Fogel (1964) and Wildenberg (1970) suggest that mitotic recombination is characterized by multiple events in the *his1* region as well. This is true of both induced and spontaneous mitotic recombination, Although the extensive co-conversion of adjacent loci characteristic of the induced mitotic recombination in the *SUP6* region is not seen as strongly in *his1*, meiotic co-conversion is also less frequent in this region (Elizabeth Savage, unpublished results from this laboratory).

The problem of variability in the frequency of gamma ray-induced prototrophs was never solved. The results of Witkin (1966) and Bridges et al. (1967) on "mutation frequency decline" in *E. coli* after UV irradiation suggest that the repair of suppressors and other genes involved in protein synthesis may be more sensitive to the physiological state of the cell than repair of other loci. Mutation drequency decline is seen as a reduction in the induced mutation frequency of suppressor mutations with no change in survival, when protein synthesis is inhibited after UV irradiation. Witkin (1966) hypothesized that the apparent specificity for suppressor mutations may reflect a specificity of the conditions required to produce a repressed state for genes involved in protein synthesis. She suggests that a gene not being transcribed is better able to be repaired.

In a review article by Hawthorne and Leupold (1974) reference is made to the work of Wyssling (1972) on mapping two suppressor loci in *Schizosaccharomyces pombe* with methylmethanesulfonate. However, no details are given on the reproducibility of the results and their sensitivity to pre- and post-irradiation conditions.

Hybrid Endings, Excision Endings and Marker Effects

The relationship between the two classes of selected prototrophs recombinant for outside markers was used as a basis for evaluation and interpretation of the quantitative mapping procedures. The $R_1:R_2$ relationship gave a map with no internal inconsistencies and oriented this map with respect to outside markers. In addition, this method separated one of the parameters of recombination (hybrid endings) from the others (Whitehouse and Hastings 1965).

Because of the non-significant difference between R_1 and R_2 in cross *1-3* x *AC*, it is possible that the order of these two alleles is reversed. The other five crosses with non-significant $R_1:R_2$ differences were crosses of non-adjacent alleles and consequently their positions could be confirmed by their behavior in combination with other alleles. As the only internally consistent map, much of the analysis and discussion is based on the order provided by this method. Absolute confirmation of allele order would of course require sequencing of the mutant tRNAs.

From the viewpoint of models of recombination, if hybrid DNA covered only one allele, and if it were resolved as a crossover, prototrophic recombinants would be of the R_1 class only. If hybrid DNA covered both sites, then only independent correction of the two alleles would generate intragenic recombinants. This is true for the models of the Meselson and Radding type (1975) as well as for the early models of Whitehouse (1963) and Holliday (1964). Equal numbers of R_1 and R_2 recombinants are expected when both alleles are included in the hybrid, and $\frac{2R_2}{R_1 + R_2}$ is a measure of the proportion of crossover hybrid DNA which covered both sites. This is called the site coefficient by Whitehouse and Hastings (1965), and it demonstrates that a relatively small proportion of hybrid endings between the alleles is sufficient to generate a significant $R_1{:}R_2$ inequality. For example, if only 20% of the selected intragenic recombinants resulted from hybrid DNA endings between the alleles, by this model we would expect to see 60% R_1 and 40% R_2.

In *SUP6* the site coefficient varied with crosses and ranged from 0.30 to approximately 0.92. From the above discussion, it follows that 8% to 70% of allelic recombination in this locus resulted from hybrid endings. This is in contrast to the situation in the *his1* locus of *Saccharomyces cerevisiae* where Fogel and Hurst (1967) found that over 90% of *his1* prototrophs with outside markers recombined were R_1. Even disregarding the reciprocal intragenic recombinants, the site coefficient for the alleles they tested was 0.15.

In certain situations, evidence of hybrid endings may also be found in selected prototrophs with parental outside markers. The frequency of the P_1 class is a measure of the frequency with which non-crossover hybrid DNA covered the proximal site plus one-half the frequency with which both sites were covered. P_2 results from non-crossover hybrid covering the distal allele and one-half the double-site frequency. A significant excess of one parental class over the other indicates that hybrid DNA entered the gene preferentially from one end and often covered one allele and not the other. Although for the majority of crosses in *SUP6* the contribution of hybrid endings to recombination was sufficient to generate a significant difference between the two recombinant classes, for most crosses no significant inequality of the parental classes was observed. In Figure 3 the inequality of the two parental classes was compared with the inequality of the two recombinant classes for each cross. Since increases in R_1/R were not accompanied by deviations in P_1/P from 0.5 (indicative of an excess of one or the other parental class), there is no evidence for hybrid DNA having entered the locus predominantly from one direction.

One feature of this figure was the high R_1/R values for all crosses involving allele *1-20*, the distal-most allele. From the above discussion of site coefficients, it may be supposed that the high proportion of R_1 recombinants indicates that a large part of recombination between *1-20* and other *SUP6* alleles resulted from hybrid endings. It is not known whether this was a consequence of long hybrid lengths which entered the gene from the proximal end with a high probability of ending between alleles *1-9* and *1-20* or short lengths which entered from the distal end. There was a slight tendency in crosses involving allele *1-20* for P_2 to exceed P_1 when R_1 was high. This suggests that short lengths of distal hybrid were responsible for the high R_1/R. If crossover and non-crossover hybrid have similar characteristics, then a preferential conversion of the distal marker among non-crossover prototrophs suggests that hybrid entered distally.

Another characteristic of the $R_1 : R_2$ relationship is that it was highly correlated with the number of intervals between the alleles.

It is seen in Figure 9 that the greater the span between the markers, the higher was the proportion of R_1 products. The number of intervals refers to the R_1:R_2 map given in Figure 2. Although it is highly unlikely that all allelic positions are equidistant, and consequently that intervals between two adjacent sites are the same length, this is taken to be a better measure of distance than the prototroph frequency. The R_1:R_2 map, because of its internal consistency, is thought to give the most reliable allele order available for this locus.

The correlation between R_1/R and the number of intervals between the alleles is significant at the 1% level ($r = .573$; df = 46) and is greater than the correlation between R_1/R and the prototroph frequency ($r = .288$; df = 46; $p = .5$).

If recombination between two alleles requires that a length of hybrid DNA ends between them or that an excision-repair (correction) length covers one allele but not the other, then the fact that the ratio of hybrid endings to excision endings changed with distance (measured in the number of intervals between them) indicates that the frequencies of hybrid and excision endings are not both directly proportional to the distance between the alleles. Since the proportion of prototrophs resulting from hybrid endings appears to have been relatively lower over short distances than over long ones, the nearby heterozygosity of closely-placed markers may have affected the formation, distribution or stability of heteroduplex. Holliday (1964) first suggested that the cause of distance dependency in allelic recombination was the inhibition of pairing by the mutant sites themselves. Ahmad and Leupold (1973) suggested that mismatches could affect hybrid stability, and they used

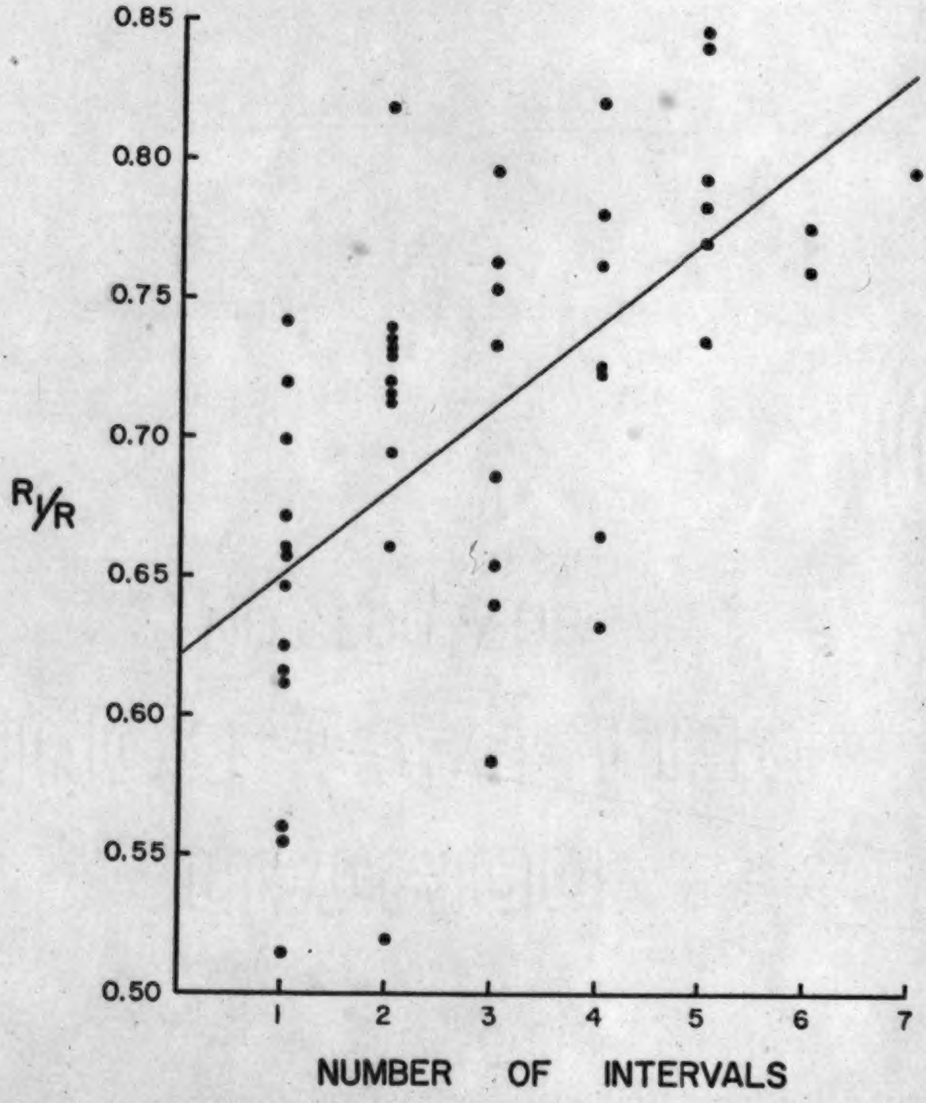

Fig. 9. Relationship between the number of intervals between the alleles and the proportion of recombinant prototrophs which were R_1. The line represents the least-squares linear regression line.

this as a basis for a model of map expansion. Holliday (1974) discussed the possibility that branch migration is impeded by mismatching. It has also been postulated (Hastings 1975) that a site of heterozygosity can draw out hybrid DNA to cover itself, thus increasing co-conversion at the expense of single-site conversion. Only this last hypothesis would allow for the occurrence of correction endings when hybrid endings between the alleles were absent. However, the occurrence of fewer hybrid endings per unit length over short distances than over long ones is expected to result in map expansion. No consistent map expansion was observed for crosses of *SUP6* alleles.

Because *SUP6* showed no map expansion, but rather a tendency towards map contraction (Fig 6), there is a preferable interpretation of the distance-dependent change in the relative contribution of hybrid endings to prototroph production. Contraction describes the situation in which the frequency of recombination from a to c (for the allele order a-b-c) is less than the sum of the recombination frequencies a-b and b-c. As explained in the Introduction, the Fincham and Holliday (1970) model for map expansion predicts that as soon as two alleles are farther apart than the excision-repair length of mismatch correction, they are no longer co-converted. One expects a sharp increase in the number of recombinant products due to the contribution of excision-repair endings to recombination. If, however, even the closest alleles are farther apart than the average length of correction, one enters a phase of map contraction. All intervals a-b, b-c and the longer a-c have been expanded by a constant amount, dependent on the correction properties of that system. By adding a constant to all three intervals, one loses additivity and sees map contraction. Furthermore, in this phase, it is predicted that there will be changes in the relative protortions of hybrid and excision endings because the probability of a hybrid length ending between two alleles continues to increase as the distance between the alleles increases. Simultaneously, the contribution of excision endings to recombination declines as the probability of hybrid covering both alleles decreases. The large amount of scatter in the map expansion plot for *SUP6* indicates that there were marker effects not accounted for in Fincham and Holliday's formulations. These will be further discussed.

The hypothesis that the distance between *SUP6* alleles was greater than the average excision length requires that the distribution of excision lengths was at least bimodal. If those excision-repair lengths which contributed to allelic recombination were very short, then there was another far more common class of excision lengths which resulted in co-conversion of *SUP6* alleles most of the time. While tetrad dissection indicated that approximately one-third of all conversion events which involved *SUP6* extended into an adjacent locus, it would appear that an even higher proportion of events did not stop within *SUP6*. Approximately 15% to 20% of tetrads were convertant for *SUP6*, yet intragenic recombination was in the range of 0.01% to 0.2%. Co-conversion is thought to have been responsible for the largest part of this difference.

The existence of two characteristic lengths of excision is not implausible in the light of the work of Cooper and Hanawalt (1972) on the heterogeneity of patch size in the repair of DNA in UV-irradiated *Escherichia coli*. The large patch and short patch repair are probably mediated by different enzyme systems. Although it has been shown that dimer excision in *Saccharomyces* should be thought of as a model for mismatch repair, not as the same process, the existence of an analogous system with two classes of excision lengths lends credence to this hypothesis.

There is strong evidence that the alleles in a cross do not simply detect recombination events which would have occurred in their absence, but to some extent determine the events which occur. Sites of heterozygosity may affect several different parameters of recombination.Leblon (1972a,b) found that in the b_2 locus of *A. immersus*, mutations induced by certain mutagens showed a high frequency of postmeiotic segregation, implying that the probability of correction of a site of heterozygosity is dependent on the molecular nature of that site. Furthermore, when a mutant allele with a low postmeiotic segregation frequency was put into a cross with an allele which showed high postmeiotic segregation, the second allele was converted under the influence of the first (Leblon and Rossignol 1973). Leblon also found that the probability of conversion to the mutant or to the wild-type allele was related to the mutagenic origin of the allele. In *Saccharomyces*, there seems to be less room for allele-specific marker effects on the process of correction. Conversion to mutant and to wild-type has been shown to be equal, with a few questionable exceptions (for example *met10-4* in this study). Furthermore, for the three alleles of *SUP6* studied by tetrad dissection postmeiotic segregation was low and nearly equal for all three. Therefore, at least for these alleles, marker effects are not explicable in terms of an inequality of correction in the two directions or differences in the probability of correction. However, it is possible that correction lengths are subject to the effects of certain combinations of alleles. This was the hypothesis which Stadler and Kariya (1969) used to explain the decrease they observed in recombination between two alleles when an additional site of heterozygosity was present in the cross. This hypothesis was also discussed by Holliday (1974) who used the results of Hurst, Fogel and Mortimer (1972 and unpublished) to support his argument. Their results showed a decrease in recombination frequency between two alleles in a four-point cross compared to the two-point cross. This was accompanied by an increase in co-conversion but no significant change in the total conversion frequency of the alleles. The additional sites of heterozygosity appear to have increased the correction length causing co-conversion at the expense of single-site conversion and recombination.

In studies in *Ascobolus immersus*, Paszewski et al. (1971) found that conversion of an allele in a two-point cross was more frequent than its conversion in a one-point cross. A more extensive comparison of one-point and two-point crosses by Baranowska (1970) showed that most, but not all, alleles had elevated conversion frequencies in two-point crosses. And Stadler and Kariya (1969) found that the decrease in recombination frequency between two alleles caused by the presence of an additional marker was accompanied by an increase in the conversion frequency of the alleles. These increases in conversion are better explained by marker-induced increases in the amount of hybrid covering both sites, that is, a type II marker effect discussed by Hastings (1975). In *SUP*6, allele *1-3* seems to exhibit this type of marker effect. Most crosses involving *1-3* showed a low proportion of R_1 products. Since crosses of *1-3* with alleles which mapped distal to it were not affected, one interpretation is that hybrid DNA which entered *SUP6* from the proximal end was influenced by the presence of *1-3* so that it often covered both sites. Because crosses of *1-3* with *1-6* and *1-7* showed nearly average proportions of R_1/R, it appears to have been a distance-dependent effect. Alternatively, it is possible that the paucity of hybrid endings was the result of hybrid DNA of distal origin which was inhibited from covering *1-3* and consequently the markers proximal to *1-3*. There is no data on the frequency of conversion of *1-3* in two-point crosses.

Other allele-specific effects were detected in crosses in which the prototroph frequencies deviated dramatically from the norm for that particular number of intervals (Fig. 5). Even in this highly non-additive map, *1-8* x *1-3* and *AC* x *1-9* stand out as crosses with unexpectedly low recombination frequencies, and *1-6* x *AC* is a cross which showed very high recombination. The effects may have been on hybrid formation and length, or on correction properties. If a low prototroph frequency were accompanied by a high $R_1:R_2$ ratio, this could have been the result of unusually long correction lengths in that cross. However, any conclusions drawn from a single $R_1:R_2$ ratio are open to question and analysis of specific crosses will not be pursued.

Moore and Sherman (1975), using defined mutations and known physical distances, attempted to analyze the relationship between physical distances and recombination rates. The different mapping procedures they tested showed deviations from the true physical distance which were not explicable on the basis of single mutant codons having a specific "marker effect", but required the interaction of the nucleotide sequences in the heteroallelic combinations.

Both the generalized marker effects discussed in terms of the Fincham and Holliday model for map expansion and the allele-specific or heteroallelic-combination-specific effects on recombination parameters are thought to have contributed to the immappability of *SUP6*.

In addition, strain-dependent differences in recombination may have contributed to a small extent to the non-distance dependent influences on prototroph frequency. The dissection of three different alleles of *SUP6* showed significant differences in conversion frequencies which may have been related to genetic background. However, the extent of the differences (up to approximately 35%) is insufficient to account for large effects on prototroph frequency. Furthermore, the strains used in two-point crosses were more closely related than the dissected strains.

While most fine-structure mapping in *Saccharomyces* has made use of induced mitotic recombination, there are a few meiotic maps. It is characteristic of these mapping studies that the alleles could be ordered using meiotic prototroph frequencies in two-point crosses but with large deviations from additivity. Examples are found in the *his1* locus (Fogel and Hurst 1967), *ade3* (Jones 1972) and *ade8* (Esposito 1968). Rothstein (1975) constructed a meiotic fine-structure map of *SUP3*, and again, although the map was far from additive, he found it possible to order the alleles. This indicates that the immappability of *SUP6* is not a general characteristic of suppressors in *Saccharomyces*.

The technique of principal coordinate analysis was applied to the problem of generating an order using recombination frequencies alone. The best approximation of a linear order by this technique showed considerable agreement with the map constructed from the $R_1:R_2$ ratios (Fig. 4), suggesting that there was some distance-dependency in the frequency of allelic recombination for this locus. Both the $R_1:R_2$ and principal coordinate analysis maps showed less deviation from additivity than the vast majority of permutations of the eleven markers.

Much of the above analysis was based on a simple model of hybrid DNA formation and mismatch correction. Because more recent versions of recombination models allow for more flexibility, interpretations of the results based on these models are more difficult (Sobell 1972; Meselson and Radding 1975; Wagner and Radman 1975). Even allowing for branch migration and regions of symmetrical and asymmetrical hybrid, it is still predicted that hybrid DNA endings between the alleles will give the R_1 class of recombinants exclusively and that symmetrical hybrid covering both will result in equality of R_1 and R_2. However, in the Meselson and Radding model for example, asymmetrical hybrid covering both alleles will give equality of the two recombinant classes only if preferential migration of the hybrid in one direction is not accompanied by a bias in which strand initiates the recombination event. If one allele is covered by symmetrical hybrid and the other by asymmetrical hybrid, R_1 is expected to exceed R_2, but the proportions of these two will again depend on initiation and migration preferences.

Conversion-associated Recombination of Outside Markers

Both tetrad and random spore analysis contribute information on the association between intragenic recombination and crossing-over between outside markers. In contrast to the results of Hurst, Fogel and Mortimer (1972), it was found that for *SUP6* outside marker exchange accompanied intragenic recombination significantly less than 50% of the time (Table 15). Some of this discrepancy (37% outside marker recombination in tetrads dissected for this study versus 51% in the study done by Hurst et al.) can be accounted for by the use of different outside markers. In their study, they used *his2* and *met10* as flanking markers, which this analysis placed 12.8 map units apart; *cdc14* and *met10*, used here, are separated by 7.8 map units. The presence of *cdc14* allowed the identification of crossovers in the *his2-cdc14* region as independent of conversion of *SUP6*. If the marker *cdc14* had not been used in this study and all crossover tetrads had been counted as recombinant whether or not they involved the converted chromatid, no inequality between the parental and recombinant classes would have been found.

There are several ways in which a real equality of parental and recombinant classes may appear as an inequality. Mutation and selection against certain outside marker combinations in random spores have been eliminated as factors contributing to the inequality, as was the occurrence of independent crossing-over. Selection against some types of tetrad was more difficult to dismiss, and the analysis of incomplete tetrads did not exclude the possibility that conversion of *SUP6* accompanied by a crossover is sometimes a lethal event. Independent conversion of outside markers in random spores may also change the parental: recombinant ratio by transferring recombinant events into the parental class and the converse. Since the exchange goes both ways, its effect will depend on the proportions of parental and recombinant convertants. If parental exceeds recombinant, more parental events would be lost than gained, so that the true excess of parental over recombinant would have been greater than observed.

Because the *cdc14* - *SUP6* co-convertants had a higher frequency of outside marker recombination than conversion of either site alone, it was thought that longer hybrid lengths might show a different parental: recombinant ratio intragenically as well. There is precedent for thinking that crossover and non-crossover hybrid may have different length characteristics. Whitehouse and Hastings (1965), in an analysis of Murray's data on the *me-2* locus of *Neurospora crassa* (1960, 1963), found evidence suggesting that crossover hybrid more often covered both alleles than did non-crossover hybrid. However, because *SUP6* lacks polarity, there is no way of separating recombinants which resulted from long hybrid lengths from those which came from short hybrid. There was no correlation between the proportion of prototrophs recombinant for flanking markers and the $R_1:R_2$ ratio, the position of the alleles, or the distance between the alleles (measured in the number of intervals). The observed correlation with prototroph frequency is not a useful one since prototroph frequency is itself a compound of several factors.

Sigal and Alberts (1972), in a study on the stereochemistry of a Holliday (1964) type exchange, have shown that the two configurations of the crossed strand exchange are equally likely and are expected to be in rapid equilibrium. If the configuration of this exchange at the time of endonucleolytic cutting is responsible for the decision as to whether a recombination event will be parental or recombinant for flanking markers, then the two resolutions are equally probable. Whitehouse (1974), in his analysis of recombination data for the *g* locus of *Sordaria fimicola*, offered mechanisms by which inequality would be observed without altering the basic assumptions. The particular deviation seen here, which is an excess of non-crossover events, is explained by postulating that when a correction length reaches the end of a heteroduplex length, the event is always resolved into a non-crossover. Whitehouse finds support for this idea in the following observations from the *g* locus data. Aberrant 4:4 asci having no correction of heteroduplex show equality of crossover and non-crossover events; 5:3 and 3:5 asci show some excess of non-crossover events; 6:2 and 2:6 asci (having the most correction) show the greatest excess. Also, the excess of non-crossovers is greater in two-point crosses than in one-point crosses for all these classes, suggesting that conversion at the second site could cause the event to become a non-crossover event.

In this study, the frequency of outside marker recombination in chromatids showing postmeiotic segregation was 46%. This is 10% higher than the amount of reciprocal recombination associated with conversion. While the bias is in the expected direction, the sample was small and the difference was not significant. Unlike what was observed in *Sordaria*, the frequency of outside marker recombination associated with recombinants selected in two-point crosses was slightly higher than that accompanying conversion in one-point crosses. Because the number of postmeiotic segregants was small, and because recombinants in two-point crosses were selected random meiotic products introducing more variables than just the addition of a second allele, the results obtained with *SUP6* cannot be used to support or refute the Whitehouse explanation. However, the data do show that crossover and non-crossover events are not equal in all loci in yeast, and that consequently yeast data does not support a model which requires this decision to be random.

Other Characteristics of the *SUP6* Region

The distinguishing features of this region are the high frequency of recombination events, the long hybrid lengths which characterize these events and the accompanying long correction lengths. There were several other characteristics of meiotic recombination at *SUP6* which may not fit into a theoretical framework at present but which contribute to the description of recombination at this locus, and may turn out to be correlated with some of these other features.

a. The observations on wider ratio tetrads suggest that not only did recombination sometimes involve both pairs of non-sister chromatids, but that if these were two separate events at the same site, they were not independent. The number of events involving more than one pair of chromatids at a site was significantly greater than expected on the assumption of independence. In their study in *Ascobolus immersus*, Lamb and Wickramaratne (1973) found positive, but not complete interference between two events at the same site in crosses with high conversion frequencies and negative interference for low-conversion crosses. The 7:1 and 1:7 segregations analysed separately showed negative site interference in both high- and low-conversion crosses. The studies reported here showed an excess of observed wider ratio tetrads to expected in all crosses. The number of 7:1 and 1:7 segregations was too small to tell if the excess was significant, but for the 8:0's and 0:8's, it was significant. Like the results of Lamb and Wickramaratne, this is evidence that hybrid DNA can form on both pairs of non-sister chromatids of a single bivalent and, furthermore, in the system it appears that the conditions which promote the formation of one heteroduplex at a site are also favorable for the formation of a second heteroduplex.

b. There appears to have been no crossover interference in the interval between *his2* and *met10*, that is, the occurrence of one crossover did not affect the probability of occurrence of others. Since crossover position interference has been clearly demonstrated in *Saccharomyces* by Mortimer and Fogel (1974), this is apparently a parameter which may vary within an organism as well as between organisms.

c. The occurrence of hybrid DNA in this region did not appear to be symmetrical on the two chromatids. In addition to the evidence from tetrads with postmeiotic segregation which was presented in the Results, there is the absence of any tetrad class which could have resulted from the conversion of *SUP6* in opposite directions on the two chromatids. There are other studies in yeast (Fogel and Hurst 1967; Fogel and Mortimer 1969) which suggest that hybrid DNA was present on one chromatid only or it extended much farther on one chromatid than on the other. As discussed in the Introduction, the amount of asymmetry is a parameter which varies between species and between strains of the same species (see Holliday 1974). It is not clear how much variation there is between loci within one organism, although the tetrad classes found in different yeast studies indicate that hybrid is consistently asymmetrical in this organism.

d. There was no negative or positive interference between conversion events in adjacent loci (excluding apparent co-conversions). The frequency of coincident but independent conversions was examined in this system because of the frequent occurrence of hybrid DNA which covered two loci. If two sites of mismatching within a hybrid are corrected independently, one expects to see a higher frequency of coincident conversions in linked genes than is predicted from the product of independent conversion frequencies. However, the detection of such independent corrections requires hybrid DNA formation on two chromatids. Independent correction of two sites on the same strand would be seen as either co-conversion or single-site conversion. In light of the strong evidence for asymmetrical hybrid, the absence of a positive correlation between conversions is not evidence against independent correction.

In this system independent correction of asymmetrical hybrid could only have been seen if the hybrid spanned three marker genes and the two outermost markers were corrected independently with no correction or correction in the opposite direction of the middle site. This type of event was not observed.

The occurrence of independent correction of two sites within a single heteroduplex is a parameter which varies with the system being studied. Stadler and Kariya (1969), using crosses segregating three sites within a gene, showed evidence for an appreciable amount of conversion in two separate segments of one chromatid with an intervening site not converted. Touré (1972), in a study of Locus *14*, a polycistronic unit in *Podospora anserina*, found such events to be very rare. Hurst et al. (1972) saw conversion of two non-adjacent alleles in a three-point cross of *arg4* alleles in *Saccharomyces*. However, in the absence of information on which strands were involved, it must be concluded that independent conversions within the locus may have been responsible for these.

e. It was noted in the Results that alleles *sup6-1-2* and *sup6-1-3* showed higher conversion frequencies than the original *SUP6-1* mutation. This may be a reflection of differences in the genetic backgrounds and/or differences in the conversion frequencies of the particular alleles. In one-point crosses involving these tow secondary *SUP6* alleles there was a coincident increase in conversion of *cdc14* and *met10*, loci which frequently co-converted with *SUP6*. There was no change in *his2*, *ura4* or *leu2* conversions. That the frequency of conversion of *met10* alone was affected as well as the co-conversions of *met10* and *SUP6* may mean that *met10* was at least partially under the influence of the same controlling elements as *SUP6* as if they shared a *cog* region, for example (see Introduction for an explanation of *cog*). The possibility also exists that an allele-specific marker effect in *SUP6* affected one of the parameters of recombination in *met10*, for example, the amount of hybrid DNA covering that allele. This would require that even conversion of *met10* alone often involved heteroduplex extending from or into *SUP6*.

The two approaches to the study of meiotic recombination in this region, random spore analysis of two-point crosses and the dissection of unselected tetrads from one-point crosses provide different information on the parameters of recombination. However, the results from both are, of course, dependent on the frequency and site of initiation and the extent of hybrid DNA as well as the properties of mismatch correction, so the two should complement one another to some extent. A few examples follow.

1) It was pointed out that tetrad dissection showed that *SUP6* alleles convert at a frequency of 15% to 20% per tetrad. Yet intragenic recombination leading to prototroph formation was 0.01% to 0.2% per spore. The difference between these is most simply accounted for by a high frequency of co-conversion, and the tetrad data on co-conversion of markers outside *SUP6* make it clear that the region is characterized by a high percentage of long conversion lengths.

2) The relationship between gene conversion and outside marker recombination is seen both in the study of tetrads and in selected random spores. Even if, as suggested earlier in the Discussion, recombinants which arose in two-point crosses resulted from an excision process with a different distribution of lengths than the excision-repair responsible for the majority of convertants, both types of event shared the property of being accompanied by less than 50% outside marker recombination.

3) The dissection of one-point crosses heterozygous different alleles of *SUP6* showed that there were no significant differences in the probability of mismatch repair and in the probability of correction to mutant or to wild-type of the different alleles. These allele-specific properties were therefore not sufficient to account for the marker effects seen in two-point crosses involving these alleles. It was therefore necessary to postulate the existence of allele- and heteroallelic combination-specific effects on other parameters of recombination.

4) The equality of 3:5 and 5:3 segregations in tetrad analysis showed that postmeiotic segregation involved a chromatid originally from one parent as often as it involved a chromatid from the other parent. It therefore appears that there was no bias as to which strand became hybrid. Consequently, although hybrid formation was not exactly reciprocal on the two chromatids, the discussion about the inequalities between R_1 and R_2 in selected random spores is still valid (see Discussion on page 103).

It is clear, then, that different approaches to the description of recombination may reveal different aspects of the process and that information available from one kind of study is capable of explaining features of another.

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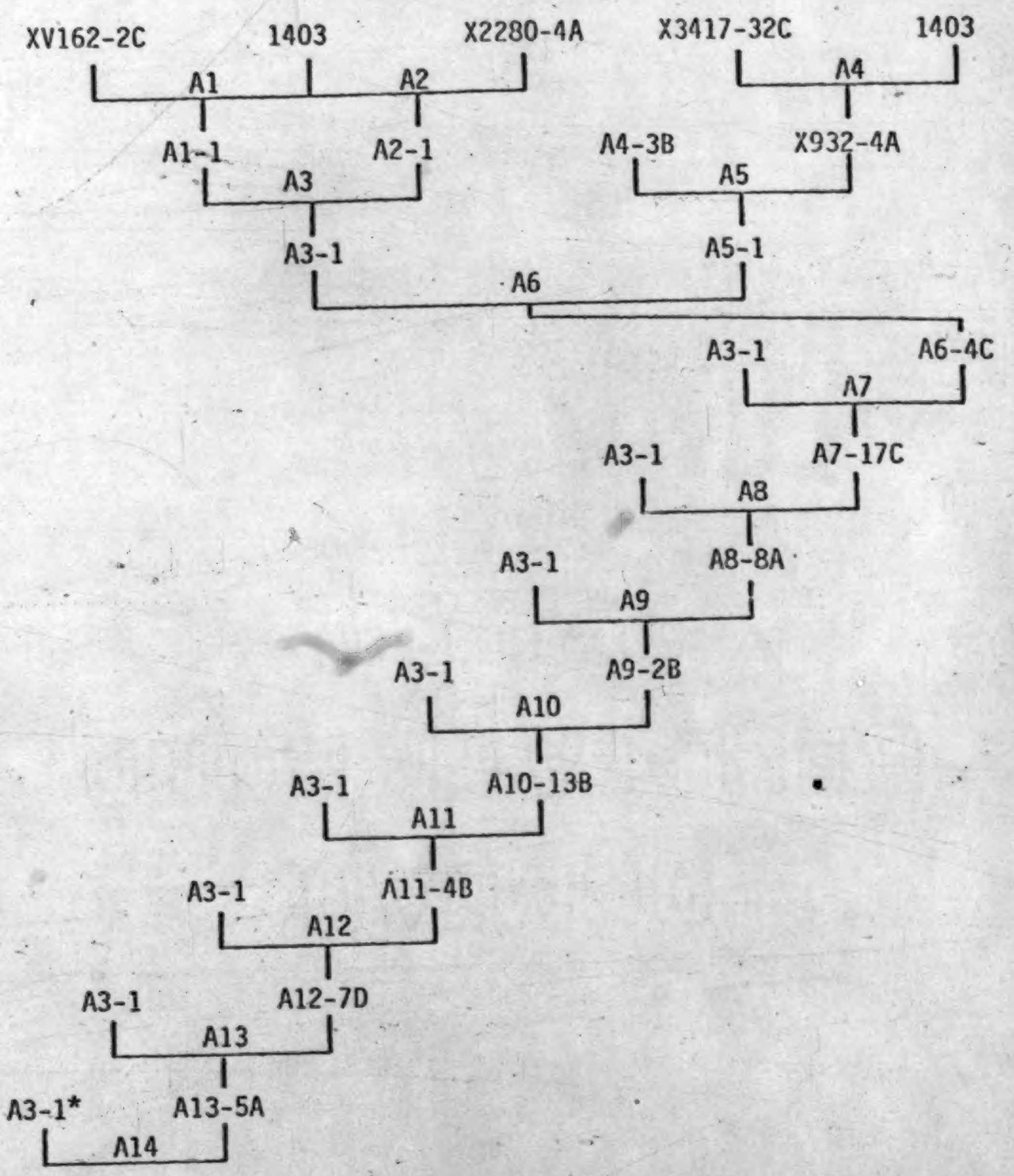
APPENDIX I

Pedigree of Strains Used in this Study

a.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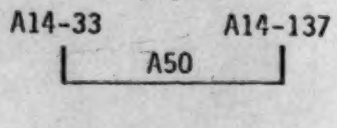
1403
X2280-4A
X3417-32C
1403
A1
A2
A4
A1-1
A2-1
A4-3B
X932-4A
A3
A5
A3-1
A5-1
A6
A3-1
A6-4C
A7
A3-1
A7-17C
A8
A3-1
A8-8A
A9
A3-1
A9-2B
A10
A3-1
A10-13B
A11
A3-1
A11-4B
A12
A3-1
A12-7D
A13
A3-1*
A13-5A
A14

* Carrying *leu2* mutation induced by UV irradiation to 10% survival

(Cont'd)

b.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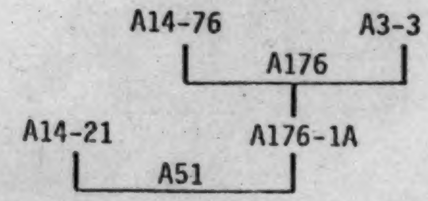
A14-137
A50

c.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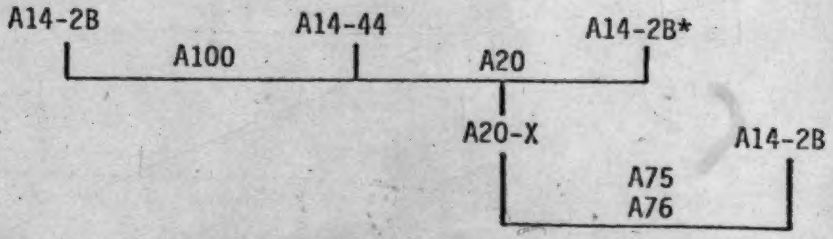
A176
A14-21
A176-1A
A51

d.


A14-2B
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A20
A20-X
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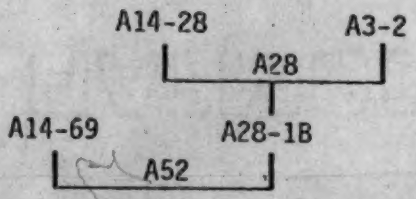
A75
A76

* Carrying a secondary mutation in *SUP6-1*

e.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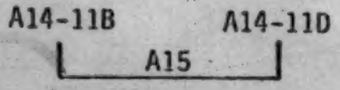
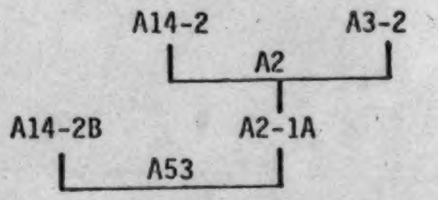
A28
A14-69
A28-1B
A52

f.


A14-11B
A14-11D
A15

(Cont'd)

g.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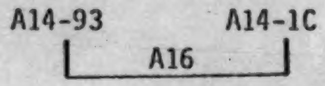
A3-2
A2
A14-2B
A2-1A
A53

h.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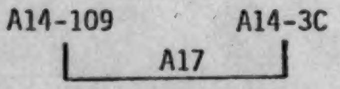
A16

i.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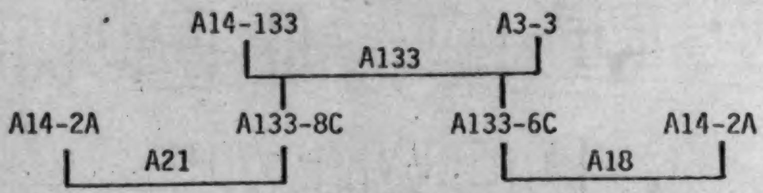
A14-3C
A17

j.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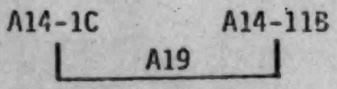
A3-3
A133
A14-2A
A133-8C
A133-6C
A14-2A
A21
A18

k.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A14-11B
A19

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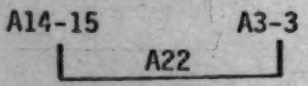
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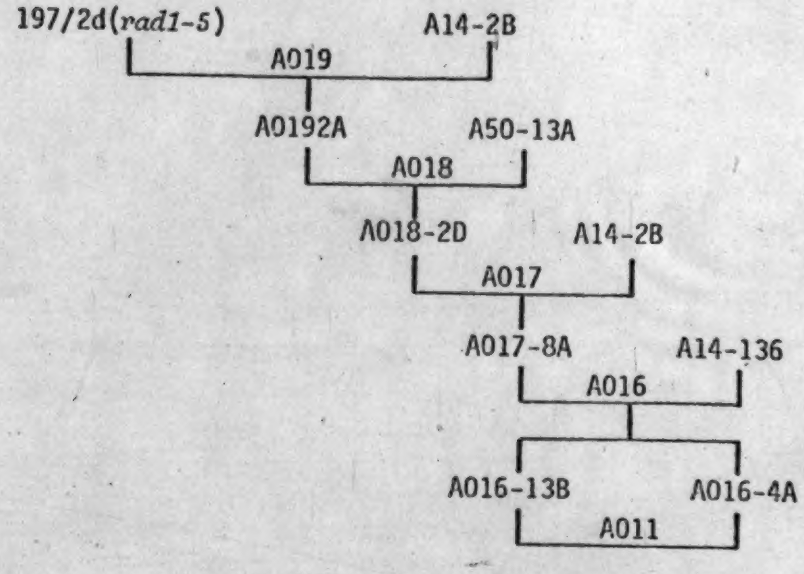
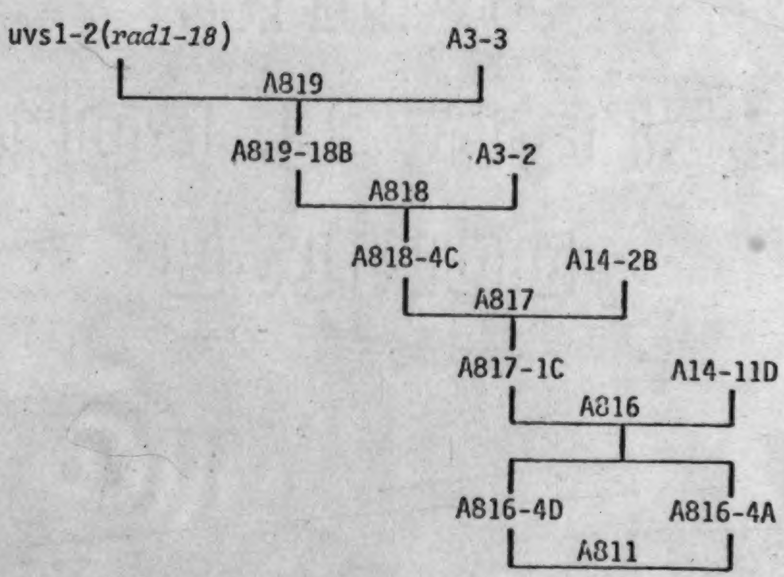
A3-3
A22

(Cont'd)

m.

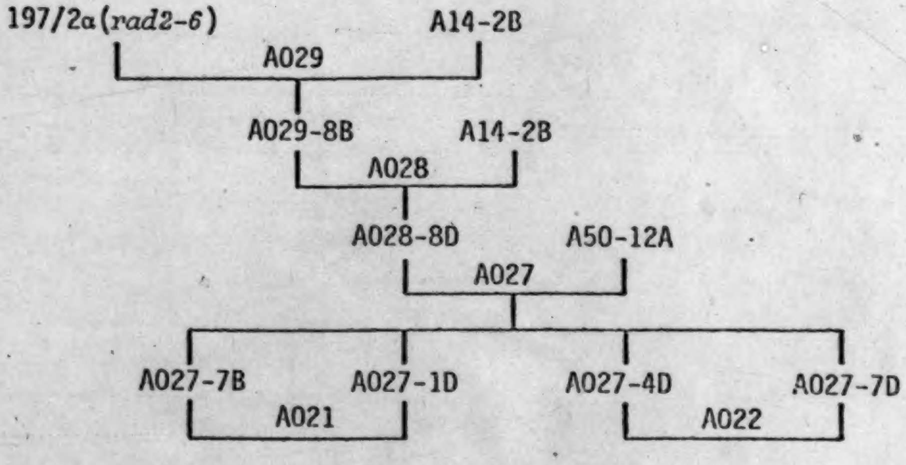

197/2d(rad1-5)
A14-2B
A019
A0192A
A50-13A
A018
A018-2D
A14-2B
A017
A017-8A
A14-136
A016
A016-13B
A016-4A
A011

n.


uvs1-2(rad1-18)
A3-3
A819
A819-18B
A3-2
A818
A818-4C
A14-2B
A817
A817-1C
A14-11D
A816
A816-4D
A816-4A
A811

(Cont'd)

o.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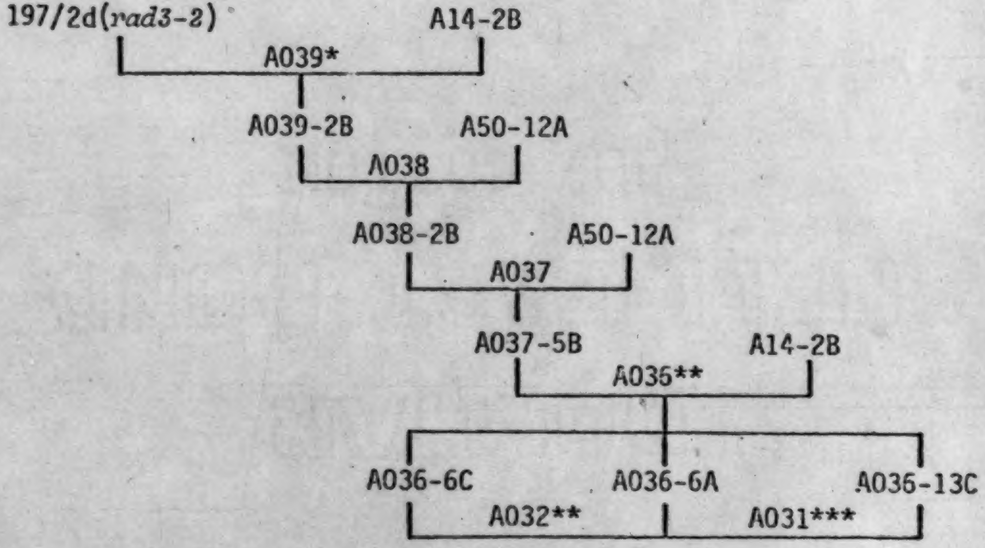
A029-8B
A14-2B
A028
A028-8D
A50-12A
A027
A027-7B
A027-1D
A027-4D
A027-7D
A021
A022

p.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A14-2B
A039*
A039-2B
A50-12A
A038
A038-2B
A50-12A
A037
A037-5B
A14-2B
A036**
A036-6C
A036-6A
A036-13C
A032**
A031***

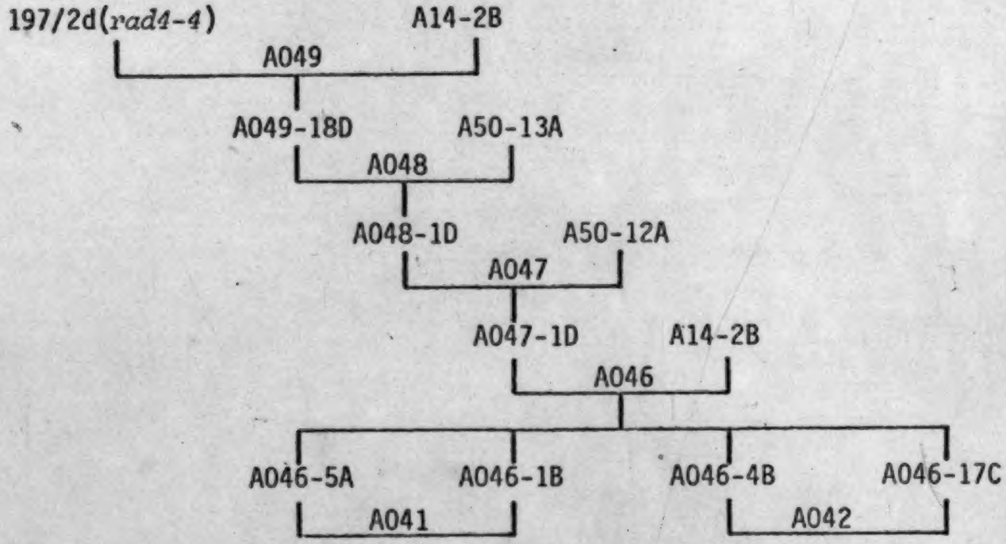
* High spore lethality. *trp5-48* suppressed in the absence of *SUP6-1*

** *ade2-1* and *can1-100* partially suppressed in the presence of *SUP6-1*

*** *can1-100* not suppressed in the presence of *SUP6-1*

(Cont'd)

q.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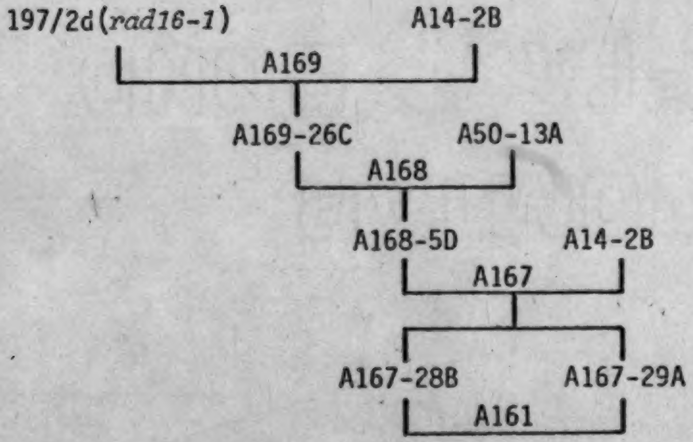
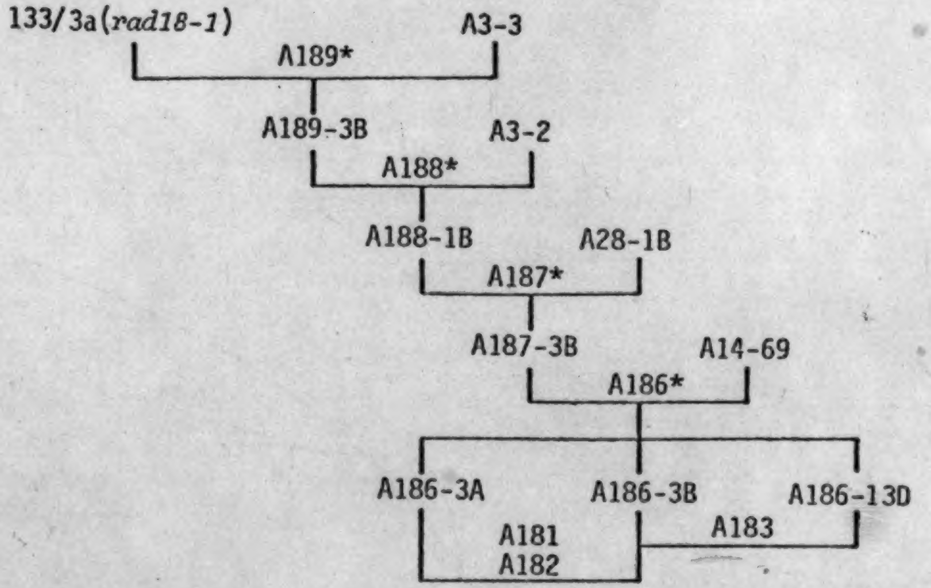
A049-18D
A50-13A
A048
A048-1D
A50-12A
A047
A047-1D
A14-2B
A046
A046-5A
A046-1B
A046-4B
A046-17C
A041
A042

r.


197/2d(*rad16-1*)
A14-2B
A169
A169-26C
A50-13A
A168
A168-5D
A14-2B
A167
A167-28B
A167-29A
A161

s.


133/3a(*rad18-1*)
A3-3
A189*
A189-3B
A3-2
A188*
A188-1B
A28-1B
A187*
A187-3B
A14-69
A186*
A186-3A
A186-3B
A186-13D
A181
A182
A183

* *trp5-48* suppressed in the absence of *SUP6-1*. The degree of suppression of *ade2-1* and *can1-100* in the presence of *SUP6-1* was variable.

Appendix II

Description of asci from diploids of the genotype

$$\frac{\text{his2 cdc14 + +}}{\text{+ + SUP6-1 met10}}\ ^{a} \quad \text{or} \quad \frac{\text{his2 cdc14 sup6-1-x +}}{\text{+ + SUP6-1 met10}}\ ^{b}$$

Number of asci from the following classes of diploids

	RAD^{+} [a] A50 A51 A52 A53	rad1-18[a] A811	rad1-5[a] A011	rad2-6[a] A021 A022	rad3-2[a] A031 A032	rad4-4[a] A041 A042	rad16-1[a] A161	rad18-1[a] A181 A182 A183	sup6-1-2[b] A76	sup6-1-3[b] A75	TOTAL
NO CONVERSION											
++-- --++ ++-- --++ [1]	278	118	119	188	136	105	116	149	183	173	1565
single exchange											
++-- --++ -+-- +-++	18	6	6	13	19	2	8	14	12	13	111
++-- --++ --+- ++-+	14	0	4	6	4	7	2	2	12	11	62
++-- --++ ---- ++++	2	2	2	3	3	2	0	2	5	2	23
multiple exchanges											
--++ -+-- +--- ++++	1	1	0	0	0	0	0	0	0	0	2
++-- --++ -+++ +---	0	0	0	0	0	0	0	0	1	1	2
-+-- +-++ -+-- +-++	1	0	0	0	0	0	0	0	0	0	1
++-- --++ ---+ +++-	0	0	0	0	0	0	0	0	0	1	1
++-- -+++ +-++ ----	0	0	0	0	1	0	0	0	0	0	1
--++ -+-- +-+- ++-+	0	0	0	0	0	0	0	1	0	0	1
++-- --++ +-+- -+-+	0	0	0	0	0	0	0	1	0	0	1
--++ ++-+ +++- ----	0	0	0	0	0	0	0	0	0	1	1
CONVERSION OF SUP6											
++-- --++ ++-- ---+	13	2	4	6	6	5	5	8	11	10	70
++-- --++ --++ +++-	9	2	6	8	5	5	3	5	10	4	57
++-- --++ ---- ++-+	4	3	2	0	1	2	1	8	6	8	35
++-- --++ ++-- ++++	3	3	1	1	6	4	1	4	2	8	33
++-- --++ -+-- +--+	1	0	0	1	0	0	2	1	1	0	6
--+- --+- ++-+ ++++	0	1	0	0	0	0	0	0	0	0	1

	RAD+	rad1-18	rad1-5	rad2-6	rad3-2	rad4-4	rad16-1	rad18-1	sup6-1-2	sup6-1-3	TOTAL
CONVERSION OF SUP6 (cont'd)											
++-- +-++ -+++ --+-	0	0	0	0	0	0	0	1	0	0	1
++-- ---+ --+- ++-+	0	0	0	0	0	0	0	1	0	0	1
++-- +--+ -+-+ --+-	0	0	0	0	1	0	0	0	0	0	1
--++ -+-- +-+- ++++	0	0	0	1	0	0	0	0	0	0	1
--++ -++- +--- ++++	0	0	0	0	0	0	0	0	1	0	1
++-- --++ ++-- +++-	1	0	0	0	0	0	0	0	0	0	1
--++ --+- ++-+ +++-	0	0	0	0	0	1	0	0	0	0	1
++-- --++ -++- +-++	0	0	0	0	0	1	0	0	0	1	2
--+- --+- ++-+ ++++	0	0	0	0	1	0	0	0	0	0	1
--++ -+-- +-++ +++-	0	0	0	0	1	0	0	0	0	0	1
++-- -+-- +-++ ---+	0	1	0	0	1	0	0	0	0	0	2
++-+ ++-+ --+- ----	0	0	0	0	1	0	0	0	0	0	1
++-- --++ -+++ +-+-	0	1	0	0	0	0	0	1	0	0	2
SUP6 4:0 OR 0:4											
++-- ---+ ++-+ ----	0	1	0	0	0	0	0	1	0	1	3
++-- ++-- ---+ ---+	0	0	0	0	0	0	0	0	0	1	1
--++ --++ +++- +++-	0	0	0	0	0	0	0	0	1	0	1
--++ --+- +++- ++++	0	0	0	0	1	0	0	0	1	0	2
POSTMEIOTIC SEGREGATION OF SUP6											
++-- --++ --++ +-P+	0	0	0	0	0	0	1	0	0	0	1
++-- --++ ++-- +-P+	0	0	0	1	0	0	0	0	0	0	1
++-- --++ ++++ --P+	0	0	0	1	0	0	0	0	0	0	1
++-- --++ -+-- ++P+	0	1	0	1	0	0	0	1	0	0	3
++-- --++ --++ ++P-	1	0	0	1	0	0	0	1	0	1	4
++-- --++ --P- --P+	0	0	0	1	0	0	0	0	0	0	1
++-+ ++-+ --P- --P-	0	0	0	1	0	0	0	0	0	0	1
++-- --++ --++ ++PP	1	0	0	0	0	0	0	0	0	0	1
++-- --++ +-++ --PP	1	0	0	0	0	0	0	0	0	0	1
++-- --++ ++-- -+P-	1	0	0	0	0	0	0	0	0	0	1
++-- --++ ++-- --P+	2	1	0	0	0	0	0	0	0	1	4
++-- --++ ++-- -+P+	1	0	0	0	0	1	0	0	0	1	3
++-- --++ --++ ++P+	1	0	0	0	1	0	0	0	0	0	2
++-- --++ +PP+ -+PP	1	0	0	0	0	0	0	0	0	0	1
++-- --++ ++-- PPP+	1	0	0	0	0	0	0	0	0	0	1
++-- --++ ++-+ -+P-	0	0	0	0	0	1	0	0	0	1	2

				RAD+	rad1-18	rad1-5	rad2-6	rad3-2	rad4-4	rad16-1	rad18-1	sup6-1-2	sup6-1-3	TOTAL
POSTMEIOTIC SEGREGATION OF SUP6 (cont'd)														
++--	-+--	+-++	PPP+	0	0	0	0	0	1	0	0	0	0	1
++--	--++	++++	--P-	0	0	0	0	0	0	0	0	0	2	2
--++	-++-	++++	--P-	0	0	0	0	0	0	0	0	0	1	1
++--	--+-	+-++	++P-	0	0	0	0	0	0	0	0	0	1	1
++--	--++	++--	--P-	0	0	0	0	0	0	0	0	0	1	1
++--	--++	-+++	+-P-	0	0	0	0	0	0	0	0	0	1	1
++--	--++	++-+	--P-	0	0	0	0	0	0	0	1	3	1	5
--++	--++	+-++	-PP-	0	0	0	0	0	0	0	0	1	0	1
++--	--++	++--	P-P+	0	0	0	0	0	0	0	0	1	0	1
++--	--++	---+	--P-	0	0	0	0	0	0	0	0	1	0	1
++--	--++	-+--	+-P+	0	0	0	0	0	0	0	1	0	0	1
++--	--++	--+-	++P+	0	0	0	0	1	0	0	0	0	0	1
++--	--++	+-++	-PP-	0	0	0	0	1	0	0	0	0	0	1
++--	--++	++--	PPP+	0	0	0	0	0	0	0	1	0	0	1
--++	---+	++++	++P-	0	0	0	0	0	0	0	1	0	0	1
--++	---+	+-+-	++P-	0	0	0	0	0	0	0	1	0	0	1
--++	--++	+++-	++P-	0	0	0	0	0	0	0	2	0	0	2
--+-	-- -	++P+	++P+	0	0	0	0	0	0	0	1	0	0	1
CONVERSION OF SUP6 WITH INDEPENDENT CONVERSION OF his2														
--++	--++	-+--	+++-	0	0	0	0	1	1	0	1	3	0	6
++--	--++	+-++	+++-	1	0	0	0	0	0	0	2	2	0	5
++--	--++	---+	-+--	1	1	0	0	0	1	0	0	0	0	3
++--	--++	--++	-++-	1	1	0	0	0	0	0	0	0	0	2
++--	++--	+-++	---+	0	0	0	0	1	0	0	0	0	1	2
--++	--++	-++-	+++-	0	0	0	0	0	1	0	0	0	0	1
++--	+-++	--+-	++++	1	0	0	0	0	0	0	0	0	0	1
++--	--++	+-+-	++++	1	0	0	0	0	0	0	0	0	0	1
++--	--++	+---	++-+	1	0	0	0	0	0	0	0	0	0	1
++--	----	+-++	++-+	0	0	1	0	0	0	0	0	0	0	1
++--	--++	++--	+--+	0	0	0	1	0	0	0	0	0	0	1
--++	++-+	-+--	----	0	0	0	0	0	0	0	0	0	1	1
++--	--++	-+-+	----	0	0	0	0	0	0	0	0	0	1	1

	RAD^+	rad1-18	rad1-5	rad2-6	rad3-2	rad4-4	rad16-1	rad18-1	sup6-1-2	sup6-1-3	TOTAL
CONVERSION OF SUP6 WITH INDEPENDENT CONVERSION OF his2 (cont'd)											
++-- +-++ +-++ -++-	0	0	0	0	0	0	0	0	1	0	1
--++ -+-- --+- ++++	0	0	0	0	1	0	0	0	0	0	1
--++ --+- -+-- ++++	0	0	0	0	0	0	0	1	0	0	1
CONVERSION OF SUP6 WITH INDEPENDENT CONVERSION OF cdc14											
++-- --++ ---- +--+	1	0	0	0	0	0	0	0	0	0	1
++-- --++ -++- ++++	1	0	0	0	0	0	0	0	0	1	2
++-- --+- -+++ ++++	0	0	0	0	0	1	0	0	0	0	1
++-- ++-- -+++ -+-+	0	0	0	0	0	0	0	0	0	1	1
++-- --++ -+++ +++-	0	0	0	0	0	0	0	0	1	0	1
++-- --++ ---+ ----	0	0	0	0	0	0	0	0	1	0	1
++-- ---+ +-++ ----	0	1	0	0	0	0	0	0	0	0	1
CONVERSION OF SUP6 WITH INDEPENDENT CONVERSION OF met10											
++-- ++-- ---+ --+-	0	0	0	0	0	1	0	0	0	0	1
++-- --++ ++++ --+P	1	0	0	0	0	0	0	0	0	0	1
++-P --++ ++-+ ----	1	0	0	0	0	0	0	0	0	0	1
++-- --++ --+- +++-	1	0	0	0	0	1	1	0	0	0	3
--++ --++ ++-+ +++-	0	0	0	0	0	0	0	0	0	1	1
++-- --+- --+- ++++	0	0	0	0	0	0	0	0	0	1	1
++-- --++ ---+ ++-+	0	0	0	0	0	0	0	1	0	1	2
SUP6 4:0 OR 0:4 WITH CONVERSION OF AN ADJACENT LOCUS											
--++ --++ +++- ++++	0	0	0	0	0	0	0	0	1	0	1
--++ +-++ +++- --+-	0	0	0	0	0	0	0	0	1	0	1
++-- -+-- -+-+ +--+	1	0	0	0	0	0	0	0	0	0	1
++-- ---- +--+ ++-+	0	0	0	0	0	0	1	0	0	0	1
++++ ++++ ++++ ++++	0	0	0	1	0	0	0	0	0	0	1
--++ --+- +-+- +++-	0	0	0	0	0	0	0	1	0	0	1
++-- ++-+ -+-- ---+	0	0	0	0	0	0	0	1	0	0	1
--++ --++ --+- +++-	0	0	0	0	0	0	0	1	0	0	1
++-- ++-- -+-+ ---+	0	1	0	0	0	0	0	0	0	0	1

	RAD^+	rad1-18	rad1-5	rad2-6	rad3-3	rad4-4	rad16-1	rad18-1	sup6-1-2	sup6-1-3	TOTAL
CO-CONVERSION OF SUP6 AND cdc14[2]											
++-- --++ ++-+ -+--	3	1	0	1	1	4	0	1	4	7	22
++-- --++ ++-- -+-+	0	2	1	1	0	1	0	1	2	3	11
++-- --++ --++ +-+-	3	0	0	1	1	1	0	2	0	3	11
++-- --++ --+- +-++	4	0	0	0	0	2	0	4	0	2	12
+-++ -+-- --+- +-++	0	0	0	0	0	0	0	0	1	0	1
++-- --+- -+-+ ++-+	0	0	0	0	0	0	0	0	0	1	1
++-- -+-- ---+ ++++	1	0	0	0	0	0	0	0	0	0	1
++-- ++-- -+-+ --+-	0	0	0	0	0	0	0	0	0	1	1
++-- --++ -+-- -+-+	0	0	0	0	0	0	0	0	1	0	1
++-- --++ -+-+ ++-+	0	0	1	0	0	0	0	0	0	1	2
++-- -+-- +-++ ++-+	0	1	0	0	0	0	0	0	0	0	1
CO-CONVERSION OF SUP6 AND met10[2]											
++-- --++ -+++ ++++	0	0	0	0	0	0	0	0	0	1	1
++-- --++ --++ ++++	1	0	0	0	1	0	0	2	2	2	8
++-- --++ ++-- ----	1	0	0	0	0	0	0	1	1	3	6
++-- --++ --++ +++P	0	0	0	1	0	0	0	0	0	0	1
--++ --++ -+-- ++++	0	0	0	0	1	0	0	0	0	0	1
CO-CONVERSION OF SUP6 his2 AND cdc14[2]											
++-- --++ --++ --+-	1	0	0	1	0	0	0	0	1	1	4
++-- --++ ++-- ++-+	1	0	0	0	1	0	0	0	1	0	3
--++ --+- --+- ++-+	0	0	0	0	0	1	0	0	0	0	1
--++ --++ --+- +++-	0	0	0	0	0	1	0	0	0	0	1
++-- --++ ++-+ ++-+	0	0	0	0	0	1	0	0	0	0	1
++-- --++ --+- --+-	0	0	0	0	0	0	0	0	0	1	1
++-- ++-+ +-++ -+--	0	0	0	0	0	0	0	1	0	0	1
CO-CONVERSION OF SUP6 cdc14 AND met10[2]											
++-- --++ --++ +-++	2	1	0	0	2	1	0	1	1	0	8
++-- --++ ++-- -+--	0	0	0	0	0	1	0	0	2	1	4
++-- --++ +-++ +-++	0	0	0	1	0	0	0	0	0	1	2
++-- -+-- -+-- +-++	0	0	0	0	0	0	1	0	0	0	1
++-- ++-- -+-- +-++	0	0	0	0	0	0	0	0	0	1	1
--++ --++ -+-- +-++	0	0	0	0	1	0	0	0	0	0	1

	RAD^+	rad1-18	rad1-5	rad2-6	rad3-3	rad4-4	rad16-1	rad18-1	sup6-1-2	sup6-1-3	TOTAL
CO-CONVERSION OF SUP6, his2 cdc14 AND met10											
++-- ++-- ++-- ++--	0	0	0	1	0	0	0	0	0	0	1
--++ --++ --++ --++	0	0	0	1	0	0	0	0	0	0	1
++-- --++ ++-- ++--	0	1	0	0	0	1	0	1	1	3	7
++-- --++ --++ --++	0	0	0	0	0	0	0	0	3	0	3
CONVERSION OF his2											
++-- --++ --++ -+--	17	6	10	11	11	8	4	19	19	16	121
++-- --++ ++-- +-++	12	7	5	15	19	10	7	10	9	10	104
--++ --+- -+-- ++-+	1	1	0	0	1	0	0	1	1	0	5
++-- +-++ +-++ -+--	1	2	0	1	1	0	0	0	0	0	5
--++ -+-- -+-- +-++	0	0	0	1	0	0	0	1	0	0	2
--++ --+- -+-- ++++	0	0	0	0	0	1	0	0	0	0	1
++-- --++ -+++ ----	0	0	0	0	0	1	0	0	0	0	1
++-- --++ -+-+ --+-	0	0	0	0	0	0	0	0	0	1	1
++-- --++ +-+- ++--	0	0	0	1	0	0	0	0	0	0	1
++-- --+- +--+ ++++	0	1	0	0	0	0	0	0	0	0	1
his2 4:0 OR 0:4											
++-- ++-- +-++ +-++	2	0	0	0	0	1	0	0	0	2	5
--++ --++ -+-- -+--	0	0	0	0	1	0	0	1	1	0	3
--++ --+- -+-- -+-+	0	0	0	0	0	1	0	0	0	0	1
CONVERSION OF cdc14											
++-- --++ --++ +---	4	1	0	0	1	1	1	1	0	2	11
++-- --++ -+-- ++++	3	0	1	0	2	1	0	2	0	2	11
++-- --++ ---- +-++	1	1	0	0	3	1	1	3	0	1	11
++-- --+- -+-+ ++++	1	0	0	0	0	0	0	1	0	0	2
++-- --++ ++-- -+++	2	0	0	1	2	0	1	3	0	7	16
--++ +++- ---+ +---	0	0	1	0	0	0	0	0	0	0	1
+-++ -+-- -+-- ++++	0	0	0	0	0	0	0	1	0	0	1
++-- --+- -+++ ++-+	0	0	1	0	0	0	0	0	0	0	1
--++ -+-- +--- +-++	0	0	0	1	0	0	0	0	0	0	1

	RAD$^+$	*rad1-18*	*rad1-5*	*rad2-6*	*rad3-3*	*rad4-4*	*rad16-1*	*rad18-1*	*sup6-1-2*	*sup6-1-3*	TOTAL
CO-CONVERSION OF *his2* AND *cdc14*											
++-- --++ ++-- ++++	1	0	0	0	0	1	0	0	3	0	5
++-- --++ --++ ----	0	0	0	0	0	0	0	1	2	1	4
--++ -+-- +-++ ----	0	1	0	0	0	0	0	0	0	0	1
CONVERSION OF *met10*											
++-- --++ --++ ++-+	8	1	0	0	2	4	0	3	6	5	29
++-- --++ ++-- --+-	3	3	1	2	0	3	0	3	3	3	21
++-+ --++ ++-+ --+-	0	0	0	1	0	0	1	0	1	0	3
++-- --++ ---+ ++++	1	0	0	0	0	0	0	0	0	0	1
++-- --++ ---- +++-	0	0	0	1	0	0	0	0	0	0	1
++-- --++ -+-+ +-++	1	0	0	0	0	0	0	0	0	0	1.
++-- --++ -+-- +-+-	1	0	0	0	0	0	0	0	0	0	1
--++ -+-- +-++ ++-+	0	0	0	0	1	1	0	0	1	0	3
++-- --+- -+-- +-++	0	0	0	0	0	0	0	0	0	1	1
++-- --++ ---+ ++-+	0	0	0	0	1	0	0	0	0	0	1
+-++ +-++ -+-+ -+--	0	0	0	0	1	0	0	0	0	0	1
INDEPENDENT CONVERSIONS OF *his2* AND *met10*											
++-- --++ --++ -+-+	0	0	0	1	0	0	0	0	0	0	1
--++ -+-+ --+- ++-+	1	0	0	0	0	0	0	0	0	0	1
++-- --+- --+- -+--	0	0	0	1	0	0	0	0	0	0	1
++-- --++ --+- -+--	0	0	0	1	0	0	0	0	0	0	1
--++ --++ -+-- ++-+	1	0	0	0	0	0	0	0	0	0	1
++-- --++ +-++ ++-+	0	0	0	0	0	0	0	0	1	1	2
'INDEPENDENT CONVERSIONS OF *his2* AND *cdc14*											
++-- --++ -+-- -+++	1	0	0	0	1	0	0	0	0	0	2
++-- --++ +--- +-++	0	0	0	0	1	0	0	0	1	0	2
--++ --++ -+-- +---	0	0	0	0	0	0	0	1	0	0	1

	RAD$^+$	*rad1-18*	*rad1-5*	*rad2-6*	*rad3-3*	*rad4-4*	*rad16-1*	*rad18-1*	*sup6-1-2*	*sup6-1-3*	TOTAL
INDEPENDENT CONVERSIONS OF *cdc14* AND *met10*											
++-- --++ -+++ ++-+	0	0	0	0	0	1	0	0	0	0	1
++-- --++ --+- +---	0	0	0	0	0	0	0	0	0	1	1
++-- --++ +--+ ---+	0	0	0	0	0	0	0	0	0	1	1
++-- ---+ -++- +++-	0	0	0	0	0	0	0	0	0	1	1
--++ +-++ ++-+ ----	0	0	0	0	0	0	0	0	1	0	1
++-- --++ -+-+ ++++	0	0	0	0	0	0	0	0	1	0	1
INDEPENDENT CONVERSIONS OF *his2*, *cdc14* AND *met10*											
--++ --++ -+-- +--+	0	0	0	1	0	0	0	0	0	0	1
POSTMEIOTIC SEGREGATION NOT INVOLVING *SUP6*											
++-- --++ ++-- -P++	1	0	0	0	0	0	0	0	0	0	1
++-- --+- +-++ PP++	0	0	0	0	0	0	0	0	1	0	1
++-- --++ +-++ -P--	0	1	0	0	0	0	0	0	0	0	1

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[1] For *SUP6*, + refers to the wild-type phenotype, without suppression
- refers to the suppressor phenotype

[2] Includes asci with independent conversion of another locus

APPENDIX III

The induction of mitotic recombinants by gamma irradiation.

Alleles	Prototroph frequency x 10^8 for doses:					Slope	y intercept	Correlation coefficient for
	0 Krad	3 Krad	6 Krad	9 Krad	12 Krad	Prototrophs/10^8/Krad	prototrophs x 10^8	prototroph frequency vs. dose
1-7 x *1-7*	15	30	45	83	118	9	4	.986
1-7 x *1-2*	15	244	377	578	646	53	53	.987
	67	1260	2330	3420	4550	361	139	.999
	67	514	1070	1730	2480	199	8	.996
1-7 x *1-6*	4260	5070	5300	5570	6160	143	4412	.975
	325	633	1240	1710	2260	165	243	.996
1-7 x *1-8*	119	1120	2160	3160	4480	372	0	.998
	25	160	317	410	468	38	49	.985
	16	326	664	945	1170	74	44	.996
1-7 x *1-10*	19	251	447	753	847	72	27	.996
1-7 x *1-11*	13	497	1010	1601	2190	182	27	.999
	35	591	1320	2150	3180	262	115	.994
	15	417	672	999	1310	106	49	.998
1-7 x *1-5*	18	132	224	379	504	41	8	.997
	4	42	63	124	278	21	24	.921
	22	262	449	585	718	57	65	.994
	21	98	196	340	304	27	30	.949
1-7 x *AC*	34	391	666	1160	1410	118	27	.996
	47	368	698	962	1420	112	31	.996

(Cont'd)

Alleles	0 Krad	3 Krad	6 Krad	9 Krad	12 Krad	Slope	y intercept	Correlation coefficient
1-7 x *1-3*	116	641	1400	1670	2620	201	82	.989
	29	197	369	493	752	58	20	.993
	23	99	175	277	409	32	7	.992
	10	267	538	819	1040	87	13	.999
	21	98	196	340	304	27	30	.949
	44	957	174	274	361	27	27	.994
	9	146	319	456	602	50	7	.999
	18	117	324	396	486	41	25	.984
	3	95	168	203	288	23	15	.991
	7	35	923	154	246	20	13	.982
	31	116	164	278	364	28	25	.994
	4	116	185	268	340	27	18	.996
	25	86	180	319	395	32	6	.992
1-7 x *1-9*	59	944	2217	2908	-	327	59	.994
	13	449	854	1239	1880	151	18	.995
	11	257	482	725	864	73	33	.996
1-7 x *1-20*	59	751	1570	1950	2690	215	112	.995
	65	1085	1705	2740	3970	316	196	.994
1-2 x *1-2*	6	34	69	64	86	6	14	.948
1-2 x *1-6*	9	62	96	149	173	14	15	.993
	7	41	65	97	115	9	11	.995
	5	44	101	164	260	21	11	.987
1-2 x *1-8*	24	26	58	73	85	6	19	.972
	9	72	132	108	189	13	23	.932
	11	19	30	47	76	5	5	.967

(Cont'd)

Alleles	0 Krad	3 Krad	6 Krad	9 Krad	12 Krad	Slope	Y intercept	Correlation coefficient
1-2 x *1-10*	20	64	62	92	145	9	21	.953
	6	19	43	58	71	6	6	.995
1-2 x *1-11*	48	95	163	238	250	18	49	.983
	11	36	40	89	118	9	6	.970
	2	6	19	18	23	2	3	.936
1-2 x *1-5*	12	144	266	406	550	45	8	1.00
	3	170	333	461	564	47	24	.995
	4	76	117	185	290	23	2	.988
1-2 x *AC*	3	148	280	373	432	36	31	.986
1-2 x *1-3*	3	65	180	207	219	19	20	.949
	15	81	203	260	408	32	0	.990
	7	77	144	260	333	28	3	.995
1-2 x *1-9*	123	664	1150	1520	1900	147	189	.996
	16	279	492	676	1040	82	12	.993
	46	361	696	943	1210	97	70	.998
	47	938	1350	2210	3450	269	11	.987
1-2 x *1-20*	11	187	329	443	593	47	29	.998
	15	256	328	485	590	46	59	.984
	15	354	571	853	891	75	87	.976
1-6 x *1-6*	5	3	4	5	8	6	5.13	.988
1-6 x *1-8*	17	103	127	180	244	18	28	.987
1-6 x *1-10*	6	76	167	191	385	29	9	.962
1-6 x *1-11*	43	206	309	508	671	52	36	.996

(Cont'd)

Alleles	0 Krad	3 Krad	6 Krad	9 Krad	12 Krad	Slope	Y intercept	Correlation coefficient
1-6 x *1-5*	14	106	189	265	347	28	19	.999
	15	179	390	553	623	53	34	.988
1-6 x *AC*	30	362	616	882	1199	95	46	.999
1-6 x *1-3*	22	310	561	851	1150	93	20	.999
	16	148	432	360	504	40	54	.921
1-6 x *1-9*	72	1270	2280	3000	4090	325	189	.997
	44	605	1120	1400	2070	161	79	.994
1-6 x *1-20*	36	535	790	1167	1823	140	29	.988
1-8 x *1-8*	40	77	120	167	210	14	37	.999
1-8 x *1-10*	8	43	62	100	105	8	13	.980
1-8 x *1-11*	20	129	164	149	164	10	64	.804
1-8 x *1-5*	42	120	304	420	685	53	3	.983
1-8 x *AC*	45	155	342	506	747	59	8	.993
1-8 x *1-3*	15	123	329	344	547	43	15	.979
1-8 x *1-9*	78	516	958	1260	1510	120	142	.992
1-8 x *1-20*	47	266	465	707	872	70	53	.999
1-10 x *1-10*	12	25	43	87	103	8	5	.980
1-10 x *1-11*	13	57	121	158	228	18	9	.996
1-10 x *1-5*	18	244	415	597	1040	80	16	.980
1-10 x *AC*	65	358	457	628	1040	74	66	.975
1-10 x *1-3*	16	120	410	283	368	29	66	.820

(Cont'd)

Allele	0 Krad	3 Krad	6 Krad	9 Krad	12 Krad	Slope	y intercept	Correlation coefficient
1-10 x *1-9*	8	300	529	666	930	74	45	.994
1-10 x *1-20*	29	475	819	1030	1390	109	93	.993
1-11 x *1-11*	20	43	39	57	59	3	25	.924
1-11 x *1-5*	21	112	210	258	295	23	40	.980
1-11 x *AC*	32	196	314	524	678	54	25	.997
1-11 x *1-3*	22	152	266	409	529	42	22	.999
1-11 x *1-9*	4	362	653	821	958	79	86	.980
1-11 x *1-20*	174	322	452	657	961	64	131	.984
	24	442	642	962	1340	105	51	.995
1-5 x *1-5*	2	22	36	68	80	7	1	.991
1-5 x *AC*	32	107	200	265	267	21	49	.964
1-5 x *1-3*	22	118	333	345	503	40	26	.976
	25	228	420	474	593	46	72	.978
1-5 x *1-9*	24	219	378	625	764	63	25	.997
	77	343	646	826	1050	81	103	.996
1-5 x *1-20*	39	442	922	1200	1930	151	0	.990
AC x *AC*	15	30	210	180	305	24	2	.933
AC x *1-3*	18	175	309	464	345	32	73	.871
AC x *1-9*	2	35	68	101	157	13	2	.992
AC x *1-20*	26	174	443	596	791	65	15	.996
1-3 x *1-3*	2	9	18	2	39	3	1	.989

(Cont'd)

Alleles	0 Krad	3 Krad	6 Krad	9 Krad	12 Krad	Slope	y intercept	Correlation coefficient
1-3 x *1-9*	24	526	818	1150	--	122	79	.992
1-3 x *1-20*	34	493	1500	1150	1480	119	221	.870
1-9 x *1-9*	27	72	95	152	228	16	18	.981
1-9 x *1-20*	37	304	493	658	1040	79	34	.989
1-20 x *1-20*	37	141	183	214	245	16	66	.957